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A preliminary report on plants treated with the carcinogenic agents of animals[1]

MICHAEL LEVINE[2]

(WITH PLATE 5)

Numerous reports are now available on the effects of various gases, vapors, and fumes on the structure of plants. Sorauer, (1922) on the basis of observation and experimental studies, lists a group of such substances, by-products of industrial activity, which affect the structure of plants. Sorauer found that hydrochloric acid and chlorine cause the death of leaves of the oat and spruce and that the structure of the leaves killed by these gases is different from that of leaves killed by other means. Discoloration and death of leaves have been reported as the common cause of fumes of hydrofluoric acid, nitric acid, and ammonia. Kny, according to Sorauer, has shown that dilute ammonia solutions injure the assimilatory activity of the plant, causing the leaves of greenhouse plants to blacken. He found that only the tissue immediately adjoining the veins of the leaves remains green.

Tar and asphalt fumes arising from street paving have been reported as harmful to such plants as the rose, strawberry, and chestnut. The common effects of the fumes of these substances are the blackening of the leaves, leaf roll, and finally death. Sorauer points out that only the surfaces of the leaves become altered. Injury of the middle of the leaf causes severe leaf roll. Tar fumes acting on *Ampelopsis quinquefolia* for a few weeks produce curling of the young green leaves, while the inner surface of the leaves becomes wrinkled by the development of the tissue lying between the finer ramifications of the veins. Cork-covered surfaces were noted near the mid-rib. With continued injury, the death of the parts of the leaves ensues and perforations follow. Young branches become corky and existing air roots dry up. Attempt at recovery has been noted, when the fumes are discontinued, by the development of corky structures. Sorauer concluded that injuries due to gaseous bodies may be of a chronic or an acute nature. In the former case the organ affected can remain alive by its successful reaction to the stimulus. In the latter case there is a rapid death of the tissue.

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It is evident that the gaseous substances reported by Sorauer in most instances prove toxic and result in the death of the most sensitive organs of the plant. The cork formation reported in mild cases indicates a type of reaction which under more favorable conditions would probably result in active cell proliferation.

The effects of ethylene and illuminating gas on plant growth have been amply reviewed and are of passing interest in so far as the present report is concerned. More recently Denny (1926)[1-2] tested 224 different chemicals, and approximately 3000 separate experimental lots were used in selecting suitable concentrations and periods of treatment, to determine their effect on the dormancy of the potato. He found ethylene chlorhydrin and the thiocyanates of sodium and potassium gave the best and most consistent results in reactivating the dormant tubers. According to Denny, McCallum suggested ethyl bromid, ethylene dichlorid, carbon tetrachlorid, ammonia, bromine and gasoline as means of arresting the dormant period of the potato.

Wallace (1926–27–28) exposed twigs to ethylene gas for from 2 to 48 hours under a bell jar and then permitted them to remain under the bell jar filled with air. He showed after a period of time, varying from a few days to several weeks, the formation of intumescences, the destruction of buds, the swelling of the apices and internodes on *Pirus Malus*, *P. ioensis*, and *Ginkgo biloba*. Intumescences which develop on buds and on stems in *Transparent* apple pass through three fundamental changes in the tissue; namely, the solution of the walls, hypertrophy of cells, and proliferation of cells. Another significant observation made by Wallace is that the faintest trace of ethylene, 1 part of ethylene to 100,000,000 parts of air, is sufficient to stimulate the production of intumescences.

In studying the effects of fungicides on leaves of the cauliflower, von Schrenk (1903–1905) found the development of numerous wart-like growths within a week, after spraying with various copper salts, in which ammonia was present. Smith (1917) investigating the mechanism of crown gall formation, after inoculations with *Bacterium tumefaciens*, concluded that the products of the metabolism of the parasite were responsible for the cellular proliferation of the host. These products of metabolism Smith believed to be ammonia, alcohol, and an acid. He believed that tumor growth in plants and presumptively cancer in man and animal are due to the physical changes induced by chemicals, such as aldehyde, acetone, alcohol, acids, alkalies, that are thrown into the cells and diffuse from them as the result of the metabolism of the feeble parasite or symbiont, together with the resultant counter-movements of water and food.

For a long time workers in anilin and paraffin factories and those employed as chimney sweeps have been subject most frequently to cancer of the skin. Coal tar and its products have been suspected of this activity. It was not until the now well-known studies of Yamagiwa and Ichikawa (1916) on the effects of coal tar painting on the rabbit and the studies of Tsutsui (1918) on the mouse, that coal tar was recognized as the irritant responsible for these epithelial proliferations known as tar cancer. The numerous publications relating to tar cancer have been thoroughly classified, and reviewed by Woglom (1926). Seelig and Cooper (1933) have continued this classification and evaluated the significance of the tar studies up to the present year. Here it is only essential to point out that repeated paintings of the skin of the ear of the rabbit or the back of the neck of the mouse with coal tar will produce cancerous growths which prove fatal to the animal. The tar acting as an irritant causes epilation, epithelial destruction, wart formations and finally, in a small percentage of animals, epitheliomas.

Recently Kennaway (1930) has shown that the synthetic hydrocarbon, 1:2 5:6 dibenzanthracene is an active carcinogenic agent producing tumors much more rapidly than coal tar; while Cook, Hewett and Heiger (1932–33) have shown that benzpyrene, another synthetic product, is probably a still more active tumor-producing agent.

The attempt to produce tumors on plants by painting with these carcinogenic agents of animals has not been attempted so far as I have been able to determine from the available literature. Komuro (1930–31) immersed seedlings of *Vicia faba* and *Pisum sativum* in a solution of coal tar for a short period of 7½ to 10 minutes. He observed vacuolization of the cytoplasm and nuclei, and giant cell formation; he also noted abnormalities in chromosome number. As indicated above, plant physiologists have studied the effects of various gases and solutions of them in air and water on the growth of the plant. Smith attempted to find a chemical substitute for the plant-tumor-producing parasite he discovered. The reaction of plants to various chemicals described by recent plant physiologists suggested the need of a more directed effort to test the influence of the known carcinogenic agents on plants.

Again, it seems of importance to produce, if possible, some type of cancer or cell proliferation in plants, which would have some provocative agent in common with a known type of animal cancer. It seems evident from the present literature that plants subjected to substances like ethylene and ammonia, produce only limited growths of abnormal cellular proliferations. It must be borne in mind that while crown gall may become

a malignant plant disease and present some analogies to animal cancer, the writer (Levine, 1931) pointed out that in any group of experimentally-induced crown galls, the large majority of them are only benign wart-like growths.

MATERIAL AND METHODS

To study the reaction of plants to paintings of coal tar, Scharlach red and 1:2 5:6 dibenzanthracene, a large variety of plants were used: *Helianthus annuus*, (sunflower); *Ricinus communis*, (castor bean); *Nicotiana tabacum*, var. *Burley*, (tobacco); *Datura stramonium*, (Jimson weed); *Solanum lycopersicum*, (tomato); *Solanum melongena*, (egg plant); *Rosa sp.* var. *Van Fleet* (rose); *Salix fragilis*, (crack willow); *Bryophyllum calycinum; Opuntia Keyensis; Carnegiea gigantea*, (tree cactus).

The annuals used in these experiments were comparatively short-lived approximately five to seven months. At about May first, seedlings were set out in the garden at Montefiore Hospital, devoted to these studies. Potted plants of the cactus species and Bryophyllum were imbedded in the earth at the same time. Cuttings of the willow had been set out in the previous year.

The carcinogenic agents were suspended in ether, pentane, or olive oil. Suspension varied in concentration from 0.1% to 10%. In a large number of cases, the concentration of the suspensions used was similar to that applied in animal-tar studies. The suspensions were painted on the apical area of the plant or in regions where the growth is usually active. The painting was made with a camel's hair brush on one side of the stem, and the same area was repeatedly painted at stated intervals. In some experiments with Ricinus and Datura, solutions were injected into the lumen of the stem. Paintings were generally preceded by slight injuries similar to those made when inoculating with *Bacterium tumefaciens*. Plants painted without previous injury were also studied. A large series of plants was painted once, while an equally large series was painted once weekly and still another series was painted three times a week.

Control studies were made on a comparatively large group of plants. Some were inoculated with *B. tumefaciens*, others were injured only in the usual manner. Since the suspension of the carcinogenetic agents was made in ether and pentane, a comparatively large number of plants was injured and painted with ether or pentane. These plants were painted as often as those which were painted with tar or Scharlach red.

A solution of 1 part of strong ammonia in 10 parts of distilled water was also used to contrast the effects of this substance with various concentrations of the carcinogenic agents. Approximately 300 plants with

935 painted or injected stems or petioles were studied. I am reporting at present on the gross effects produced by these carcinogenic agents. Plants with single or two or more paintings were fixed in preparation for a microscopical examination of the tissues. In a subsequent communication, a more detailed statement will be made regarding the microscopic changes observed.

OBSERVATIONS

The following report deals with the effects of painting localized areas, or injecting into various organs of growing young plants the carcinogenic agents, coal tar, Scharlach red, or 1:2 5:6 dibenzanthracene. The procedure used, followed in general the methods employed in producing tar tumors on rabbits and mice, modified by the method generally employed in producing plant tumors by the use of *B. tumefaciens*.

Multiple paintings with coal tar and Scharlach red

Paintings on sunflower, Bryophyllum, castor bean, tobacco, Jimson weed, crack willow and cactus were started early in June and continued for a period varying from one to five months. Paintings on the Bryophyllum and cactus are still in progress in the greenhouse. The paintings were made in some cases once a week, while in other series of studies three weekly paintings were given. The area chosen was generally the apical or growing portion of the plant. The rapidity of growth of the sunflower and its early maturity offer a splendid opportunity to study this plant in detail in a comparatively short time.

Painting the apical rosette of the sunflower, at the time the plants have attained a height of 3 to 4 feet, with a 10% solution of tar in ether results (fig. 1) in the dwarfing of the shoot. The internodal spaces remain short; the margins of the new leaves are somewhat altered. This has been observed in all the plants studied. Repeated, and in many cases, single paintings of the apical part of the growing shoot, result in the dwarfing and finally in the death of the shoot.

When the painting with 10% tar or 1% Scharlach red in ether has been confined to the regions below the growing point, normal development is interfered with in this area. The growth, instead of being positive heliotropic, becomes lateral (fig. 1). In some cases this lateral growth is of short duration. It seems that the distal cells not affected by the paintings, continue their growth normally and the stem straightens out and becomes negative geotropic again. In many cases the growth of the painted area is lateral while the new growth of the terminal portion of the plant is vertical. The lateral growth of the painted area, it appears, is due to the

more rapid growth of the unpainted surface. There is marked swelling of the painted area (figs. 3, 4, 5) and thickening of the tissues about the injuries made by the needle. The swellings become noticeable in four to five days, for the punctures made are prominent at first, and are soon surrounded by what appears to be young callus tissue. This growth is rapid and the small hole made by the needle is closed, giving the appearance of wart-like structures frequently observed in woody twigs after inoculation with *B. tumefaciens*. In non-injured but painted stems, a smooth thickening is noted (fig. 2) above the area painted, while the painted region itself appears smaller in diameter.

Cross and longitudinal sections of sunflower stems injured and painted once weekly for two months or more show increased thickness of the wood and cortex while the pith is much reduced in size. The part of the plant penetrated by the needle becomes surrounded by a thick layer of woody tissue which is continous with the thickened wood of the stem.

Coal tar (10%) and Scharlach red (1%) suspended in ether or pentane produced similar results on the plants treated, although the majority of the plants painted with ether and the carcinogenic agent show greater reaction than those painted with pentane in which the animal-cancer-producing substance was suspended.

Painting with ether or pentane alone, preceded by injury, interferes with the growth of the apical portion of the stem but the reactions are comparatively smaller than those produced with tar or Scharlach red. There is a slight callus formation about the injured tissue slightly in excess of the control stem in which the tissue was punctured 5 to 10 times with a sterile needle. Stems of sunflower (fig. 11), Ricinus and Datura injured with a needle previously dipped in a sub-culture of an active strain of *B. tumefaciens* produce the well-known crown galls. Stems, on which no pronounced surface scaring of hyperplastic tissue was formed after painting and injury, were observed and considered as "non-takes." Longitudinal sections of these stems revealed large masses of tissue about the injured internal areas. This is clearly shown in the Ricinus and Datura, which are characterized by large hollow stems. Paintings of tar or Scharlach red, as I shall mention below, induce the formation of overgrowths in the lumina of the internodes in the Ricinus and to a less marked degree in the hollow stems of Datura.

The effects of repeated painting of the sunflower with various dilutions of tar and Scharlach red were studied. These plants were slightly scarified over a given area about 1 cm to 3 cm in length and then painted three times a week beginning August 3rd and continued until September 23rd with 0.5% and 1% solutions of Scharlach red in ether and with 0.5%, 2%

and 4% solutions of tar in ether. Ten plants were painted in each series. Thirty-six shoots of the crack willow were also painted with the same suspensions. The latter paintings were continued to November 4th, when one or two shoots from each series were removed and photographed. Paintings are still in progress on the remaining shoots. The first three or four paintings of the willows result in destruction of the bark and cortex until the wood is exposed. Callus tissue is then formed around the injured painted areas until the wound is entirely closed. This results in a swelling of the stem over the painted area. No globular galls have been formed, although the reaction is far in excess of the controls which at this time, show cork-covered streaks in the stem where the scratches were made in the bark.

The stems of the sunflower in this series were studied after 19 paintings, approximately two months after the experiment was started. The results show that the various concentrations of the carcinogenic agents produced similar results (figs. 6–7–8). There was comparatively no difference in the size of the swellings of the stems over the painted areas. The reaction in the larger plants, however, was comparatively greater than in the small thinner shoots. Longitudinal sections through the painted portion of the stems show considerable hyperplasia of the wood tissue and in some cases small wart-like bodies were observed on the surface of the stem, comparable to poorly developed crown galls.

The pith was broken up by hyperplastic areas which extended from the cortex and wood. It is clear from these studies that the repeated paintings of tar or Scharlach red suspended in ether produced similar effects on plants; these studies show further that various dilutions of these agents are similar in their effects on plants. It is also evident that malignant growths analogous to those produced by tar on mice or rabbits are not produced on the species of plants studied, though the duration of painting was comparatively longer than that necessary to cause cancer on animals. Furthermore, the carcinogenic agents of animals do not call forth the large tumor-like growths induced by inoculations with *Bacterium tumefaciens* in plants grown under similar conditions. Nevertheless these striking reactions which result from paintings with tar and Scharlach red indicate clearly that plant tissues are stimulated to proliferation by these agents.

Weekly paintings of the cactus with 10% solution of coal tar started on June 3rd, have yielded no visible external overgrowths although depression of the painted areas was noted, after one month. The failure of these plants to react promptly is expected in view of the fact that crown galls artificially induced in these plants develop very slowly as shown in the studies on *Carnegiea gigantea* (Levine, 1933) and *Opuntia Keyensis* (in press).

The leaves of fourteen plants of Bryophyllum, young and old, were painted either with 10% coal tar in ether or 10% coal tar in pentane. The leaves turned brown, showed leaf roll, and died after a month. In another series of tests with this species the stems of 20 plants were painted with 10% coal tar or 1% Scharlach red in ether. The latter agent produced swellings or tumor-like growths on the stems which are still in the process of being painted. The effects of the coal tar are not as striking as those induced with Scharlach red.

Single paintings with coal tar and Scharlach red

Early in the studies of the effects of tar and Scharlach red on plant tissues, it was noted, as mentioned above, that in some cases, a single painting often caused wilting of the injured and painted shoot, and the complete death of the organ followed. Even where the injury consisted of two or three scratches of the epidermis the painting caused death. It appeared to me that the repeated paintings destroyed many of the newly formed cells, thus preventing the formation of globular galls, and that stouter organs of the plant, such as the basal parts of newly formed shoots or well-formed petioles, would respond more favorably to a single painting. The effects of a single painting preceded by slight injuries were studied. A series of sunflowers, castor beans, tomato plants, Jimson weeds, tobaccos, and egg plants (15 of each species) were selected for this test. Ten per cent coal tar in ether and one per cent Scharlach red were used. The same method was followed as outlined above. As control, a smaller number of plants, in each group, was injured without painting and another small group of plants of the same species was inoculated with *B. tumefaciens*. The control-injured plants produced long scars as the stems elongated. The painted plants showed retardation of the area painted in less than seven days. The stem in the sunflower showed marked swelling and the edges of the points of injury became thicker. One month later all tar or Scharlach red had disappeared as far as could be seen with the naked eye. There was evidence of retardation of growth over the painted area. A slight bend of the stem at the painted area was clearly seen. This, however, had disappeared at the time the plants were photographed two months after painting. The painted surfaces of the stems were covered with a scaly, bark-like structure and small intumescences covered with a thin layer of epidermis were noted (figs. 9–10). Cross and longitudinal sections show excessive thickening of the woody structures with marked growth of wood into the pith about the areas injured. The tomato, tobacco and egg plant responded similarly but to a slighter degree; the greatest reaction occurring in the tomato and least in the egg plant. The Ricinus stem

(figs. 14–16) one month after painting is swollen and shorter. Its surface is pigmented and scaly. On making longitudinal section of the stem, the lumen of the internode painted is completely filled with new tissue, arising from the inner lining of the stem (figs. 13–15). The reaction of the Datura was like that of the Ricinus but less marked.

Single paintings with 1:2 5:6 dibenzanthracene

In mid-August a large number of plants were subjected to a single painting with a suspension of 1:2 5:6 dibenzanthracene rubbed up in olive oil. The suspensions were made approximately as follows: 10%, 2%, 1%, 0.5%, 0.1%. For these studies the sunflower, castor bean, Jimson weed, tobacco, and tomato were used. For the purposes of studying the effects of the agent on woody perennial plants, the crack willow and Rose were also used. As in the previous studies, the growing parts of the stem were used. The paintings were preceded by slight injuries such as pricking or scratching the surface of the organ with a sterile needle; then the area was covered with a suspension of the agent. Care was taken to inflict only very slight injuries. One painting was given all the plants mentioned except the sunflower which was painted twice, twelve days apart. The areas painted were rather small approximately ½cmsq to 1 cmsq.

The more concentrated suspensions produced necrosis and in some cases, as in the tobacco, caused the leaf, shoot, or petiole to break off. The development of the growing point was entirely suspended and necrosis followed, whereupon side shoots were soon formed from axillary buds. Painting with 1%, 0.5% or 0.1% also caused some necrotic area about which fresh pale green colored tissue was formed about the borders of the injuries, especially in the rose, Datura, tomato, and Ricinus. The majority of cases of the rose and willow showed cork-covered scars over the injured area with marked swellings of the painted portion of the stem (fig. 12). The sunflower and Daturas showed the greatest reaction. Here comparatively large swellings in the nature of thick callus tissue, were formed. Longitudinal sections of the Datura and sunflower stems showed the pith invaded by the pale green parenchymatous tissue. Besides these swellings of the stem and the internal formation of callus tissue no distinct globular masses comparable to crown gall were formed. The reactions, however, were in all cases much greater than in the controls.

Single injections with various agents

The hollow structure of the Ricinus and Datura stems is well adapted to injections with various agents. I have also perforated the stem and heads of the sunflower, the stems of the tobacco, and the green tomato

fruits with a large hypodermic needle, which acted as a delicate gouge, removing a fine cylinder of the tissue and thus leaving a hole or canal into which the agents in question were introduced. For these experiments 0.5%, 2%, 4%, and 10% coal tar suspended in ether were used. One part of ammonia to 10 parts of distilled water and 1% Scharlach red in ether were also used. From 0.5 cc to 1 cc of the substance was introduced by means of a fine hypodermic needle.

One hundred and forty injections were made. Twenty-four hours later it was noted that a number of Ricinus plants were severely affected; the petiole and in some instances the upper part of the stem of the plant wilted and died; this was especially noted when ammonia and the 2% ether and tar were used.

A number of tomato fruits large and small injected with a 10% or 4% solution of tar in ether, became soft, affected with mold, necrotized, and fell from the vine. A number reached maturity, colored quickly, and appeared normal except that a depression in the area of injection was present. Scar tissue was formed but no excessive gross proliferations were noted.

The injected Ricinus (figs. 17–18–19–20–21, see description of figures for explanation) and Datura stems showed extensive proliferation of the lining cells of the lumen; proliferation of the cells about the point of inoculation formed a callus which showed small intumescences. The reaction of the lining cells of the lumen in the Datura was less marked than in the Ricinus. In several instances the insertion of the needle at the point of bifurcation of the Datura stem was sufficient to cause it to split in half. The split stems opened and separated by subsequent growth. The inner surfaces which were subjected to ammonia showed a smooth layer of newly formed tissue. The new tissue is brown and has the appearance of cork although green parenchymatous tissues below the outer layer were found. In a number of cases the splitting of the stem extended only for a short distance along the stem. Here callus-like-growths appeared which tended to close the wound in the stem. This occurred in cases where the lumen was injected with tar and ether solutions.

No difference has been noted in the effects of the different solutions injected. The larger the stem, the greater appears the reaction. The absence of the characteristic nodular growths which result after *B. tumefaciens* inoculation indicate that all cells exposed respond alike; this is unlike the reaction obtained with the parasite. There is unquestionably a reaction to the stimuli but it is less marked than in the crown gall disease. Injected sunflower heads and stems and tobacco stems show apparently little neoplastic reaction. The sunflower heads turned brown and died

while the tobacco survived but formed only slight scar tissue. Abundant material from all plants studied in these experiments has been prepared for microscopic examination.

DISCUSSION

The belief, that the mere presence of the bacterial parasite is insufficient stimulus to give rise to the plant neoplasm, known as crown gall is generally accepted. Smith (1917) believed that the chemical substances, produced by the parasitic organism in its metabolic processes, are more likely the active agents. When he injected the chemicals, ammonia etc., formed by the organism in vitro and presumably in vivo, into the plant he obtained intumescences, in the lumen of the Ricinus stem. Smith contended that the ammonia remains in the hollow of the stem and offers a prolonged stimulus to the plant tissue. The action of the carcinogenic agents should be of longer duration than the ammonia. Yet the overgrowths produced in Ricinus treated with tar, Scharlach red and dibenzanthracene, appear to be only slightly greater than the reactions obtained with ammonia injections. Single or repeated surface paintings after slight injury, or single injections into the lumen of such plants as Ricinus and Datura give similar results in my studies irrespective of the concentration of the agents used.

Smith's hypothesis fails to explain why the cortex and cambium in the Ricinus, sunflower etc., painted or injected with the carcinogenic agents of animals, or injected with ammonia, fail to form a distinct globular gall on the surface of the plant similar to that produced by the crown gall organism. The neoplastic tissues resulting from the chemical irritants are developed in the tissues, especially in stems with lumina and, according to Smith, are comparable to those formed by injections of suspensions of *B. tumefaciens* in distilled water. It appears that the presence of the organism in the tissue may have some unknown function in tumor formation other than producing the irritating substances. This further separates the neoplasia of plants from cancer of man and animal. The injury used to introduce the organism of the irritant into the host tends to initiate cell proliferation. The chemicals seem to cause cell proliferation for a limited number of generations. While the causes of cancer are still unknown, cell proliferation in neoplasia of man and animal is apparently without limit.

A careful study of my material shows that the cambium or meristematic tissue of the plant responds to the carcinogenic agents and suggests further that the rate of cell proliferation is equalled by the capacity of these cells to differentiate and age so that the mature structures of the stem are rapidly formed. In the crown gall tissue, it appears, the rate of

differentiation is much slower than cell multiplications. Differentiation is completed with the ageing of the plant tumor, converting the mass of young parenchymatous cells into wood and cork. In cancer, only cell proliferation occurs, with little or no differentiation.

So far, large superficial malignant galls have not been produced in plants by chemical means comparable to those induced by *B. tumefaciens* or other parasitic fungi or nematodes. The malignancy of the neoplastic tissue formed by paintings or injections of tar, Scharlach red, 1:2 5:6 dibenzanthracene or the injections of dilute solutions of ammonia is questioned. Swellings, wart-like growths and large internal intumescences in plants have been produced by the carcinogenic agents of animals. Some very few overgrowths formed in the sunflower reported above are only suggestive of crown gall.

It appears from an analysis of the material that the optimum action of the plant to the animal carcinogenic agents may be obtained with a single painting. This leads to the conclusion reached by Leitch (22), who stated that the bias toward malignancy is probably given the cell during the preneoplastic stage. Findlay (1925) showed that mice painted once with hot tar, produced epitheliomas in 3 out of 75 cases. Helianthus and Ricinus stems painted once with tar or Scharlach red, produced, two months later, swellings and internal overgrowths that are as well developed as those painted 12 to 20 times during a similar period.

It appears that further study should give rise to new methods of chemical or physical stimulation of the plant which will result in the formation of malignant growths like those produced by *B. tumefaciens*. Such results may throw new light on the question of cell proliferation and may possibly clarify our conception of the cancer problem.

It seems, from these studies, that the reaction of plants to the carcinogenic agent of animals is limited. The protective response of the plant to the irritants employed in these experiments is greater than that reaction induced by mechanical injury, but is not so great as that induced by *B. tumefaciens*.

SUMMARY

A report is made on the gross effects of painting or injecting carcinogenic agents of animals, coal tar, Scharlach red, 1:2 5:6 dibenzanthracene on the following plants: *Helianthus annuus*, (sunflower); *Ricinus communis*, (castor bean); *Datura stramonium*, (Jimson weed); *Nicotiana tabacum*, var. *Burley*, (tobacco); *Solanum melongena*, (egg plant); *S. lycopersicum* (tomato); *Bryophyllum calycinum; Rosa* sp. var. *Van Fleet; Opuntia Keyensis; Carnegiea gigantea*, (tree cactus); *Salix fragilis*, (crack

willow). More than three hundred plants were treated with these agents suspended in ether, pentane or olive oil in different concentrations. Some plants were given a single painting while others were painted once weekly, others thrice weekly, for periods from 2 to 5 months. Plants studied were injured prior to the painting so as to simulate conditions generally employed when inoculations are made with *Bacterium tumefaciens*. A large series of plants were injected with various dilutions of tar or solutions of ammonia in water.

The paintings result in a proliferation of tissues about the injured and painted areas giving rise to swelling of the stem with small irregular masses of new tissue. Longitudinal sections of the painted stems show thickening of the woody elements, new wood tissue formation with abundance of parenchymatous tissue. Painting or injecting of plants with hollow stems show excessive proliferation of the inner lining of the stem filling the entire lumen with new tissue.

There is no difference in effects produced by single or repeated paintings; nor is there any difference in the effects of the different concentration of the carcinogenic agents used.

Control plants grown under similar conditions inoculated with *B. tumefaciens* show the usual external tumor masses typical of the crown gall disease. Plants injured and painted with ether and pentane show structural changes with slight hyperplasias. A more detailed report, fully illustrated, will follow.

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Explanation of plate

Fig. 1. Sunflower, growing point, injury followed by 7 paintings, each painting made at interval of one week, with 10% coal tar suspended in ether.

Fig. 2. Sunflower stem not injured, painted 5 times, each painting made at interval of one week, with 10% coal tar in ether.

Fig. 3. Sunflower stem as in fig. 2, injured before painting.

Fig. 4. Sunflower injured before application of 12 paintings with 1% Scharlach red suspended in ether.

Fig. 5. Sunflower stem as in fig. 4, painted with 10% coal tar suspended in pentane.

Figs. 6, 7, 8. Sunflower stems painted following injury, with ½% and 1% solution Scharlach red in ether, three paintings per week were made, total paintings 19.

Fig. 9. Sunflower stem painted once with 10% solution of coal tar in ether, painting made after injury on 7/10/33; photographed 8/15/33.

Fig. 10. Same as fig. 9, painted with 1% Scharlach red in ether.

Fig. 11. Sunflower stem inoculated with *Bacterium tumefaciens* on 6/17/33; photographed 8/7/33.

Fig. 12. Rose stem painted after injured with 0.1% of 1:2 5:6 dibenzanthracene in olive oil. Painted on 8/17/33; photographed 11/4/33.

Fig. 13. Longitudinal section of Ricinus stem painted once with 1% Scharlach red in ether, after injury, on 7/10/33; photographed 8/15/33.

Fig. 14. Surface view of Ricinus stem shown in fig. 13.

Fig. 15. Longitudinal section of stem of Ricinus painted with 10% coal tar in ether, after injury. Painted 7/10/33; photographed 8/15/33.

Fig. 16. Surface view of stem shown in fig. 15.

Figs. 17,18. Longitudinal section of two stems of Ricinus' internodal space injected on 7/31/33 with 0.5% solution of coal tar in ether; photographed 10/7/33.

Fig. 19. Same as figs. 17–18, injected 2% solution of coal tar in ether on 8/1/33.

Fig. 20. As above, injected 4% coal tar in ether on 8/2/33.

Fig. 21. As above, injected with 1 part of ammonia in 10 parts of distilled water on 8/7/33.

LEVINE: REACTIONS TO CARCINOGENIC AGENTS

UNIV. OF MICH.

The effects of x-rays on growth and respiration of wheat seedlings

DOROTHY S. FRANCIS

(WITH SEVEN TEXT FIGURES)

INTRODUCTION

The discovery of x-rays by Röntgen in 1895, followed in 1896 by the discovery of radioactivity by Becquerel and of radium by the Curies in 1898, opened a new field for research in physics, chemistry, and biology. Many investigations have been made subsequently to determine the physical characteristics of the radiations and to improve the methods of obtaining them. Researches have been conducted also to ascertain the effects produced by these radiations on matter—both non-living and living.

Radiations from radioactive substances are known to be of three main types: (1) alpha rays, which consist of helium nuclei (positively charged), ejected at high speed; (2) beta rays, consisting of electrons (negatively charged), travelling at very high speed; (3) gamma rays, which, for the present purpose, may be considered to be electromagnetic in nature. X-rays also are of this latter type of radiation, and differ from gamma rays only in wave length. All these radiations have the common property of ionizing matter and may be referred to, therefore, as ionizing radiations.

The action of such radiation on living matter may be considered to take place in three steps, of which the first is a physical effect, known as ionization. This ionization is the result of the interaction between radiant energy and matter whereby certain atoms lose temporarily some negative electricity in the form of electrons and become positive ions. The electrons thus liberated attach themselves to atoms or groups of atoms which thereby become negative ions. It should be noted that only a very small percentage of the total number of atoms in the path of the rays is ionized at any one time. Electrons may be liberated by radiation from any kind of atom; therefore, any substance may be ionized—whether it be an electrolyte or a dielectric; organic or inorganic; solid, liquid, or gaseous.

The presence of these electrically charged ions in a compound facilitates a regrouping of the atoms into new compounds. Accordingly, a secondary effect of radiation on matter is the production of chemical changes. Such changes, however, are not always readily perceptible, since only a small fraction of the total number of atoms is ionized, and any changes which take place under these conditions may be reversible.

Changes in the chemical composition and equilibria of an organism may initiate modifications in the biological processes which occur normally.

Accordingly, the production of biological changes in living matter by radiation may be considered to be a tertiary effect.

The primary effect of radiation—ionization—takes place during the time that a substance is exposed to the radiation. The secondary effect—chemical effect—being subsequent to ionization, requires a certain time for its manifestation, which may be short or long, depending on various conditions. In a complex system the first chemical change probably occurs almost simultaneously with ionization. This then may set in motion a series of chemical changes which are independent of further irradiation. The observable biological change is the end result of these chemical changes. Time is required for the completion of this process. Therefore, there should be an interval (latent period) between the exposure to radiation and the manifestation of a biological change. Furthermore, this interval should depend on the nature of the biological change under consideration, since various biological responses require different lengths of time before they become evident. Thus, in studying eye defects produced by irradiating eggs of *Drosophila* (fruit fly), one must wait until the time when eyes develop in the organism before observing the abnormalities.

From these considerations it follows that radiation may produce many biological modifications in a given test object, and that these become evident at different times after irradiation. Whether these changes are detectable or not depends on several factors, but principally on the dose of radiation administered, the radiosensitivity of the material, and the equipment available for the observation of such changes.

Numerous investigations have been reported concerning the biological action of x-rays and other ionizing radiations. Many of these are qualitative studies which describe certain effects resulting from the administration of radiations, without quantitative definition of the doses employed or the effects produced.

Cytological studies have been carried out by a number of investigators, employing either plant or animal material, or both. The results obtained by a few of these workers will serve to illustrate the general types of changes observed in irradiated material.

Among those who have studied the histological modifications produced by x-rays in plant material may be mentioned Johnson (1926), Komuro (1928, 1930a, 1930b), and Bersa (1927a). Some of the changes described included enlargements and vacuolations of the cells in the meristematic region of the root tips; an increase in the amount of xylem at the expense of the pith; and the presence of a greater amount of suberin than usual in the hypocotyl regions of mature plants. Another alteration mentioned was the formation of enlargements in the root tips of x-rayed seedlings;

these enlargements were referred to as "roentgen tumors." In these tumors were giant cells which pushed away the surrounding cells into other rows, thus producing a disturbance in the alignment of the cells. In addition, there were many cells which were multinucleated or degenerated; some of the nuclei contained double chromosome numbers, abnormal nuclear figures, attenuated nuclear membranes, and vacuolized chromatin masses. Another effect of x-rays was a temporary depression in the frequency of nuclear divisions, immediately following irradiation. This depression seemed to be due to a delay in the inception of mitosis, and not to any change in the time interval necessary to complete any one stage in mitosis. This latter type of disturbance was corroborated by observations of Strangeways and Hopwood (1926), experimenting with chick embryos *in vitro*.

Other qualitative investigations have been carried out to study genetic changes which appear in irradiated material. As representative experiments, those performed by Efroimson (1931), Goodspeed (1929), Hanson and Winkleman (1929), Patterson (1929), Painter and Muller (1929), Mavor (1929), and Stadler (1930) may be cited. Experiments of this kind have been performed on a variety of test objects, including *Drosophila melanogaster* (fruit fly), *Nicotiana tabacum* (tobacco), *Hordeum vulgare* (barley), *Zea Mays* (corn), and other materials. The appearance of mutations, the production of breaks in chromosomes, the non-disjunction of the sex-chromosomes, and the translocation of chromosomes are among the effects reported.

Qualitative experiments have been performed also to determine whether radiation alters the viscosity of protoplasm or of protein solutions. The results of these experiments are conflicting. Fairbrother (1928) reported that irradiation with x-rays brought about a marked decrease in the viscosity of egg albumen. On the other hand, Wels (1924) stated that the application of x-rays effected an increase in the viscosity of serum and globulin solutions *in vitro*. Weber (1923) could detect no change in the viscosity of the protoplasm of *Spirogyra* or *Phaseolus* (bean) after exposure to x-rays. Nadson (1925) pointed out the fact that the viscosity of the protoplasm of *Saccharomyces cerevisiae* (yeast) may decrease at first and later increase, after irradiation with beta and gamma rays of radium.

Similar discrepancies appear in studies which have been made of the effects of radiations on the hydrogen-ion concentration of body fluids. When irradiation with x-rays was severe enough to injure the cells, the reaction of blood became acid, according to Cluzet and Kofman (1925). Pannewitz (1927) reported an acid reaction immediately after irradiation of blood serum, albumen, and globulin solutions *in vitro* with x-rays; but

he stated that this acidosis might be followed by an alkalosis lasting for several days. Fernau (1925) and Kolta and Förster (1926) described an alkaline reaction in blood irradiated with x-rays. Woodard and Downes (1931), however, found no consistent change in the hydrogen-ion concentration of blood of irradiated patients, nor in the blood of rabbits after the administration of lethal doses of 200 K.V. roentgen rays.

The very recent development of quantitative methods for the study of the biological action of x-rays and other ionizing radiations has depended mainly on two factors: (1) the devising of suitable methods and instruments for accurate mensuration of the radiation employed; (2) the improvement of technique and apparatus for the measurement of biological modifications produced by radiations. Considerable emphasis should be placed upon quantitative studies in this field, for it seems certain that information regarding the *modus operandi* of the biological action of radiations may be obtained only by correlating a large number of biological changes with the dosages effecting them.

Radiation may be measured by any given effect which it is capable of producing in matter. Effects which may be utilized include the blackening of a photographic plate, the production of fluorescence in certain bodies, the change in a chemical compound, or the modification of a biological process. Ionization, however, is usually employed as the basis for the most convenient and accurate methods of measuring radiation. It is desirable, of course, to express the measurements in terms of a standard unit. Such a unit was established for x-rays in 1928, and is defined as follows: "The International Unit of x-radiation is the quantity of x-radiation which, when the secondary electrons are fully utilized and the wall effect of the chamber is avoided, produces in one cubic centimeter of atmospheric air, at 0°C. and 76 cm. mercury pressure, such a degree of conductivity that one electrostatic unit of charge is measured at saturation current." This unit is called the roentgen (r).

Recently, a number of papers have been published which give quantitative data on the biological effects of radiations. Some of the experiments reported have been designed to study the effects of varying the conditions of radiation used on the same kind of test object. Other tests have been arranged to study the effects of a constant type of radiation on varying test objects or different physiological responses. For investigation of certain problems of the first category, tests have been carried out to determine the characteristics of different qualities of radiation by comparing the effects which they produce on living matter.

As a criterion of the effect of different qualities of radiation, Packard (1927, 1928) studied the mortality produced in the eggs of *Drosophila*

melanogaster (fruit fly). He found that the mortality curve for these eggs showed the same characteristics, regardless of the quality of radiation. In 1932 he investigated the biological effectiveness of x-ray beams of wave lengths of 0.05, 0.08, and 1.70 A.U., obtained from tubes maintained at 550, 300, and 12 kilovolts, respectively. Using *Drosophila* (fruit fly) eggs and fragments of mouse tumors as test objects, he reported that equal doses of these three qualities of radiation, as measured by air ionization chambers, produced equal effects. He pointed out that this confirmed earlier experiments which he had performed with wave lengths of 0.20, 0.30, 0.50, and 0.70 A.U.

Holthusen and Zweifel (1932) performed a series of experiments on the eggs of *Ascaris megalocephala* in an endeavor to discover if there were any difference in the effects of radiations of different wave lengths on these organisms. They could detect no difference in the degree of injury produced by x-rays, gamma rays, and corpuscular rays.

Henshaw, Henshaw, and Francis (1933) investigated the effectiveness of 165-kilovolt x-rays, 700-kilovolt x-rays, and gamma rays of radium in causing growth reduction in *Triticum vulgare* (wheat) seedlings, and on the mortality of *Drosophila* (fruit fly) eggs. They reported a slight difference in the relative effectiveness of these qualities of radiation, which, however, was not definitely greater than the possible experimental error. Therefore, they stated that these results, taken alone, do not prove that there is a differential effect depending on the quality of radiation.

Lachmann and Stubbe (1932) investigated the effects of hard and soft x-rays on the growth of *Vicia faba* (Windsor bean). They found that for the lower doses (50 roentgens) the soft rays were more effective than the hard rays; for somewhat higher doses (100 or more roentgens), however, the hard x-rays were far more effective than the soft rays. At 500 roentgens the degree of injury produced by the two qualities of radiations began to be somewhat comparable, and it seemed likely that the degree of injury produced by very heavy doses of the two qualities of radiations would be about the same.

Several investigators have attempted to determine whether the effect of a certain dose of radiation on the organism is the same when the radiation is administered over a long period of time as when it is administered over a short period. In other words, do effects of radiations follow the Bunsen-Roscoe law, which states that, for a given degree of response, *intensity* $\times$ *time* = *a constant*? Packard (1926) performed a series of tests, involving the use of 50,000 eggs of *Drosophila melanogaster* (fruit fly), which showed that the Bunsen-Roscoe law was applicable within the limits of the experimental conditions employed.

Roesler and Henshaw (1932), however, found that a less marked effect was produced on *Drosophila* (fruit fly) eggs when the intensity was low and the time of exposure was long than when the same dose was administered in a short time at a high intensity. Ancel (1927), in experiments on lentil seedlings, observed a marked decrease in the effect of x-rays when, for a given dose, the duration of the exposure was increased and the intensity of the radiation was decreased.

A number of radiological studies have dealt with the influence of ionizing radiations on various biological processes. Several investigators have studied the effects of radiation on the growth of seedlings.

Bersa (1926) conducted experiments to determine the effects of x-rays on the growth of seedlings of *Vicia faba* (Windsor bean), *Sinapis alba* (mustard), and *Zea Mays* (corn). In a very few cases he obtained increased growth of the shoots following relatively weak doses of x-rays. But he could detect no corresponding increase in the growth of the root system. In general, he noted that doses as weak as 10 Holzknecht's units caused a marked decrease in the growth of the roots. With doses ranging from 10 to 40 Holzknecht's units, this depression was only transitory. After small doses inhibition of growth was of shorter duration than after large doses.

Reinhard and Tucker (1928a, 1928b) irradiated seedlings of *Vicia faba* (Windsor bean), *Pisum sativum* (pea), and other species of plants. They concluded that the sensitivity of seedlings to x-radiation depends on the species irradiated, the age of the plant when irradiated, the time of day when radiation is administered, and the portion of the plant exposed.

Injurious and retarding effects of x-radiation have been reported by Heeren (1932), who was able to measure very minute changes in the growth of *Vicia faba* (Windsor bean) by means of an interferometer. He pointed out that there was a distinct retardation in the growth of seedlings which were exposed to x-rays of an intensity of 15 roentgens per minute. From the beginning of irradiation he observed a retardation, which persisted throughout the entire duration of the irradiation. As soon as irradiation ceased, there was an increase in the rate of growth of the roots.

The development of apparatus for the accurate measurement of respiration has made possible a new method of investigating the effects of radiations on living materials. This affords an index of the influence of radiations on one of the most fundamental of physiological processes. Several studies recently have dealt with the effects of radiation on respiration. As examples of this type of experiment, those performed by Bersa (1927b) and Adler (1929 and 1930) may be mentioned. They employed different

test objects—*Vicia faba* (Windsor bean) and rat testicles—and used different methods of measurement of respiratory activity; but both reported decreased respiration after heavy irradiation of the material. After weak doses there appeared to be a transitory acceleration in some of the plant material.

Cattell (1931), working in the Biophysical Laboratory of the Memorial Hospital, developed an excellent technique for using the linear growth of the different organs of seedlings of *Triticum vulgare* (wheat) in the study of certain radiation problems. He found that this material possesses several advantages: The radiation effects on the growth of four different organs of the seedlings may be measured within a short time after the administration of radiation. The results may be duplicated from time to time with reasonable precision. Failla and Henshaw (1931) and Henshaw, Henshaw, and Francis (1933), working in the same laboratory, used this organism extensively in studying the influence which the wave length of radiation exerts on the quantitative relation between dose and effect.

Although retardation of the linear growth of the four organs of the seedlings has been found to possess considerable practical value, it must be recognized that this is only one manifestation of the effects of radiation. It is of interest, therefore, to search for other manifestations with the twofold purpose of acquiring more information about the mode of action of radiation and possibly of discovering some radiation effect which is of even greater biological significance than growth retardation.

The work which is reported in this paper was performed in the Biophysical Laboratory of the Memorial Hospital during 1931 and 1932. The author wishes to express her appreciation to Prof. S. F. Trelease, of the Department of Botany of Columbia University, for many helpful suggestions and criticisms. It is with pleasure, also, that she acknowledges her indebtedness to Drs. G. Failla and P. S. Henshaw, of the Memorial Hospital, for their interest and assistance on numerous occasions.

MATERIALS AND METHODS

The experiments reported in the present study were arranged so that four physiological responses to x-rays might be investigated in the same material—seedlings of *Triticum vulgare* (wheat). Each test covered a period of about seventy-six hours and included irradiation of the experimental seedlings, followed by periodic measurements of growth in length of the four different organs (coleoptile, leaf, primary root, lateral roots), determinations of fresh and dry weights of the growing portions, and measurements of carbon dioxide production. The process of germination was started by soaking the seeds in water. After an interval of twenty-four

hours the experimental seedlings were irradiated. Determinations of growth and respiration were made thirty hours, fifty-two hours, and seventy-six hours after the dry seeds had been put to soak.

The technique followed for germination and irradiation of the seedlings was the same for all tests. Seeds of "Kota" wheat were soaked in tap water for three hours, and then planted in glass chambers containing two thicknesses of filter paper moistened with 30 cc. of water. These chambers were placed in a dark incubator at a temperature of 26° C. for twenty-four hours. At the end of this time the seeds had germinated, the coleoptiles being about 2.0 mm. in length and the primary roots about 3.0 mm. At this stage, seedlings were selected for irradiation. Groups of about five hundred seedlings were placed in small cardboard trays which had been dipped in paraffin. Three trays at a time were covered by a filter of 2.45 mm. aluminium and 0.1 mm. copper. They were supported on a celluloid tray at a distance of 35 cm. from the target of the x-ray tube. The latter was a standard, water-cooled, Coolidge tube, and was maintained at a potential of 200 kilovolts and a current of 32 milliamperes. At this setting the output of the machine was 226 roentgens per minute. The trays of experimental seedlings were exposed to x-rays for intervals of 2½, 5, 7½, 15, 30, and 60 minutes, respectively. One tray was not irradiated, but was retained as a control.

Following irradiation, the seedlings were removed from the trays. Those to be used for the measurements of growth and weight were planted in Petri dishes containing two sheets of filter paper and 10 cc. of water. For studying respiration, groups of one hundred seedlings were placed on large glass slides covered with moist strips of filter paper. The slides were put in cylindrical chambers (35×5 cm.) that were kept in the incubator, and connected to the titration flasks of the respiration apparatus.

The study of the effects produced by the x-rays on the seedlings was begun approximately thirty hours after the seeds were soaked—that is, about six hours after irradiation. The linear growth was determined in millimeters for each of fifty seedlings of the controls and of the irradiated wheat. The growing portions of one hundred representative seedlings of each group were separated from the hard storage regions of the seed, and after removal of the excess water by blotting, they were placed in a small beaker and weighed, in order to determine their fresh weight. The same portions were then dried in an incubator at 60° C. for a period of about fourteen days, and in desiccators for nine days. Then the dry weights were obtained. As previously mentioned, determinations of growth and weight were made at intervals of thirty hours, fifty-two hours, and seventy-six hours after the seeds were soaked.

Coincidentally with the measurements described above, respiration was studied in similar groups of seedlings. The carbon dioxide production of the seedlings was used as a measure of their respiratory activity. The apparatus employed was essentially the same as that described by Mack (1930), which, in turn, was a modification of an apparatus described by Carrick (1928). The diagram in figure 1 shows the essential features of the

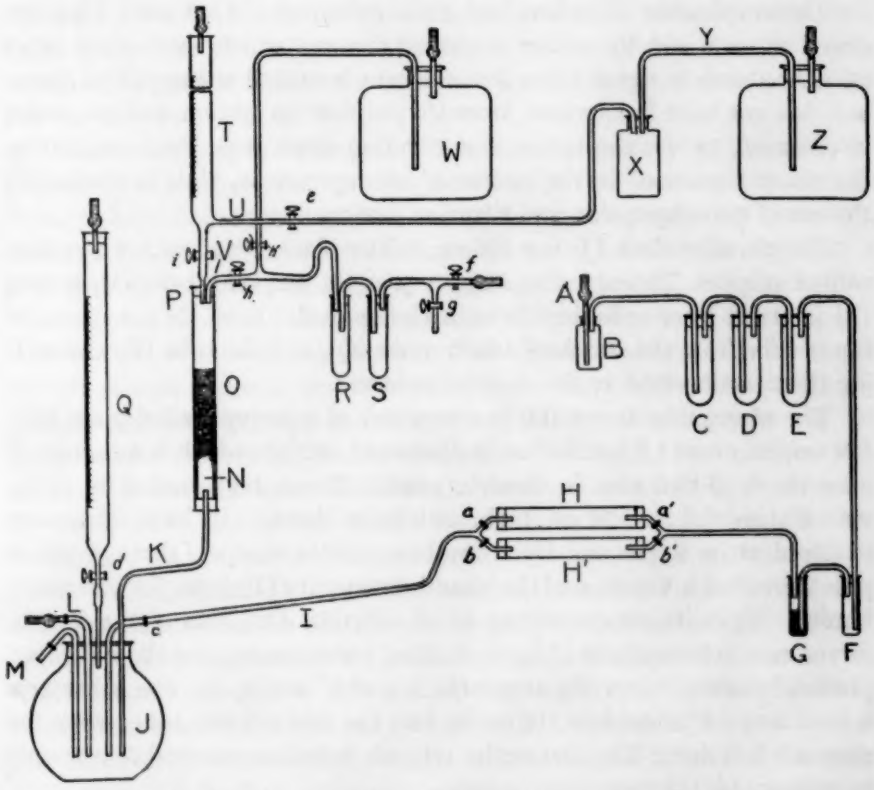

Fig. 1. Apparatus used to determine carbon dioxide production. Explanation in text.

respiration apparatus employed in these experiments. The apparatus consists of the following main parts: the purification system (A to G, inclusive); the respiration chambers (H and H′) containing the seedlings; the titration flask (J); the absorption tower (O); the outlet tell-tales (R and S); the standard acid burette (Q); the automatic burette (T) containing standard alkali solution; the 19-liter reservoir (W) of distilled water and phenolphthalein.

The purification system is designed to remove dust particles and carbon dioxide from atmospheric air, before the latter passes through the respiration chambers, and to maintain a constant humidity for the seedlings. Air from out-of-doors is passed through soda-lime (A); then through a 10 per cent solution of sulphuric acid (B); next, through a 10 per cent solution of sodium hydroxide (C, D, E); and finally, through distilled water in two bottles (F, G), one of which contains a number of glass beads.

The respiration chambers are glass cylinders (35×5 cm.) that are closed at each end by rubber stoppers, through which short glass tubes pass. As shown in figure 1, the arrangement is such that the pair of chambers has one inlet in common, from the purification system, and one outlet in common, to the absorption and titration apparatus. Four respiration chambers were used in the course of an experiment, thus necessitating the use of two absorption and titration devices.

The titration flask (J) is a 500-cc. culture flask, fitted with a five-hole rubber stopper. Through this stopper pass the gas inlet tube (I), a vent (L) guarded by a soda-lime tube, a siphon-outlet tube (M), a glass delivery tube from the standard acid burette (Q), and the tube (K) connecting the titration flask to the absorption tower.

The absorption tower (O) is composed of a heavy-walled glass tube (49 cm. long and 1.8 cm. in inside diameter), within which is a column of glass beads (3 to 5 mm. in diameter) about 20 cm. high resting on an inverted glass vial (N), 10 cm. high, with holes blown in its base. The tower is closed at its upper end by a two-hole rubber stopper, through which pass the end of a Y-tube and the glass delivery tube (P) from the automatic burette (T) containing standard alkali solution. One arm of the Y-tube serves as a delivery tube (V) for distilled water containing the indicator, phenolphthalein, when the stopcocks h and h′ are open. The other arm is bent into a U and forms the outlet into the first tell-tale tube, when the stopcock h is open. The first outlet tell-tale tube is connected to a second by means of a U-tube.

The outlet tell-tales (R, S) are test tubes (2×18 cm.) provided with two-hole rubber stoppers and inlet and outlet tubes. They are arranged so that any gas that is not taken up in the absorption tower bubbles through a solution of barium hydroxide before escaping from the apparatus. The second outlet tell-tale is provided with a Y-tube, one arm of which serves as an additional vent, guarded by a soda-lime tube; the other arm is connected by a metal tube to a suction pump which draws the gas through the apparatus. If no white precipitate appeared in the tell-tales, it was certain that no carbon dioxide had escaped from the absorption tower.

The standard acid burette (Q), of 100 cc. capacity, is used only in per-

forming titrations. During the course of an experiment the burette is disconnected from the rest of the apparatus by closing the stop-cock (d). The burette is filled with standard hydrochloric acid solution[1] from a 19-liter stock bottle, at intervals during a day's tests.

The automatic burette (T) is likewise of 100 cc. capacity. The delivery tube passes into the absorption tower at P. The lateral, U-shaped branch of the burette is connected to a long tube (U), which conveys standard barium hydroxide solution[1] from a choke-bottle (X) when the stop-cock (e) is opened; the choke-bottle is filled to a constant level by a siphon connection (Y) from a 19-liter stock bottle (Z), which is guarded by a soda-lime tube.

Before carrying out a series of tests, it was necessary to clear the apparatus of any atmospheric carbon dioxide. This was accomplished as follows: As soon as the seedlings had been placed in the respiration chambers, chamber H was clamped off by Hoffman clamps at a and a′; the stop-cock h was opened; and by opening the stop-cock g and closing f, carbon dioxide-free air was aspirated through chamber H′ (which was not clamped). This stream of air was regulated so that approximately 220 bubbles per minute passed through the absorption tower. As carbon dioxide-free air passed over the seedlings, it collected any carbon dioxide present in the chamber and carried it into the absorption tower (O), where it was precipitated as barium carbonate. Thus, in the course of a half-hour, chamber H′ was freed of any carbon dioxide which might have entered it when the seedlings were put into it. When chamber H′ was clear, it was closed at b and b′ with Hoffman clamps, while chamber H was freed of carbon dioxide. During this time carbon dioxide accumulated from the seedlings in chamber H′ and thus initiated the period of gas collection for chamber H′. An hour after chamber H′ was cleared thoroughly and closed, a Hoffman clamp was closed at c on the gas inlet pipe (I). Chamber H′ was unclamped and chamber H was clamped. Aspiration was stopped by closing the stop-cock at g. All barium hydroxide solution remaining in the absorption tower was lowered into the titration flask by opening the vent stop-cock (f). Distilled water containing phenolphthalein was washed down into the absorption tower by opening the stop-cocks h and h′. Then the solution was neutralized in the titration flask (J). In order to insure that there was no barium hydroxide solution remaining in the absorption tower, the neutralized mixture was transferred several times from the titration flask to the absorption tower. As soon as the indicator ceased to change to a red

[1] The solutions used in this work were standardized by Dr. H. Q. Woodard, of the Chemical Laboratory of the Memorial Hospital, to whom the author wishes to express her appreciation.

color, it was evident that no barium hydroxide remained in the tower. When this procedure was ended, the neutralized solution was siphoned out of the titration flask through the siphon outlet (M). Distilled water containing phenolphthalein was then discharged again into the absorption tower and titration flask. If no color change took place, it was obvious that no barium hydroxide remained either in the absorption tower or in the titration flask.

When all barium hydroxide solution had been neutralized and the apparatus had been washed and emptied, a known quantity of 0.075 N (and later, 0.127 N) barium hydroxide solution was run into the titration flask. Aspiration was recommenced by closing the stop-cock at f and opening that at g. The Hoffman clamp at c was opened, and aspiration drew the barium hydroxide solution up from the titration flask into the absorption tower, and drew the carbon dioxide from the respiration chamber through the absorption apparatus. At the end of an hour of aspirating through this chamber (H′), there had been absorbed not only the carbon dioxide produced by the seedlings while the air stream was passing through the chamber, but also all gas which had been accumulated during the hour when no air stream was passing through it (i.e., while it was clamped, during the process of clearing chamber H). In this way the total accumulation of the carbon dioxide gas produced during the interval of two hours was collected from the respiration chambers in alternate hours. At the close of each collection period the clamp at c was closed, and aspiration was stopped. The clamps were removed from the closed chamber to the open one. The vent at f was opened, and the barium hydroxide remaining in the titration flask was titrated with 0.056 N (and later, 0.071 N) hydrochloric acid solution, using phenolphthalein as an indicator. The difference between the amount of acid which would have been necessary to neutralize the original quantity of barium hydroxide, and the amount which actually was required to neutralize the remaining quantity of barium hydroxide solution, was equivalent to the amount of barium hydroxide which had been used to form barium carbonate. From this, it was possible to calculate the number of milligrams of carbon dioxide which had been produced by the seedlings during the collection period.

Determinations of the carbon dioxide production in irradiated and non-irradiated seedlings were performed at average intervals of thirty, fifty-two, and seventy-six hours after soaking the seeds. The results obtained are represented graphically in figure 4. They represent the averages of experiments involving at least three hundred seedlings—in many cases, a considerably greater number.

In order to insure reliable results, numerous tests were made of the ap-

paratus. Frequent titrations of the standard solutions were performed to check any possible changes in their concentrations. The apparatus was checked for leaks by making blank tests, with no seedlings present. Finally, the absorbing efficiency of the apparatus was tested by liberating known quantities of carbon dioxide into the titration flask and absorbing tower. The quantities recovered from a large number of tests, in which the amounts of carbon dioxide liberated were varied from 2.5 to 13 milligrams, proved the apparatus to be 100 per cent efficient under the experimental conditions.

RESULTS AND DISCUSSION

Presentation of data

The results obtained in the various experiments are summarized in tables 1 to 3, inclusive, and they are shown graphically in figures 2 to 7, inclusive. For each of the processes considered, the data are presented as percentage differences between the values for the controls and those for the irradiated seedlings.

TABLE I

Relative radiosensitivity of the several organs of seedlings at various ages and maintained at different temperatures

ORGAN	MAINTAINED AT 26°C.			MAINTAINED AT 19.5°C.	
	30 hours	52 hours	76 hours	72 hours	94 hours
Coleoptile	1.0	1.0	1.0	1.0	1.0
Leaf	1.0	1.4	1.4	1.5	1.4
Primary root	1.7	1.5	1.4	1.5	1.5
Lateral roots	3.0	1.8	1.6	1.7	1.7

The curves of figures 2 to 5, inclusive, serve to correlate the doses of x-rays administered with the degree of effect produced on the various physiological processes investigated. Since all these curves were derived in a similar manner, it will be sufficient to explain the method of obtaining the data for figure 2, which represents the percentage reduction of the fresh weights of the seedlings, as related to the dosage of x-rays. For each set of plants the difference was computed between the fresh weight of the irradiated seedlings and the corresponding weight of the non-irradiated seedlings; the value thus obtained was divided by the fresh weight of the non-irradiated seedlings, the result indicating the percentage reduction in fresh weight of the seedlings brought about by exposure to x-rays. A statistical analysis of the data showed that the results were significant.

Before discussing the results in detail, it may be well to point out that the effects produced by x-rays on the different physiological processes in-

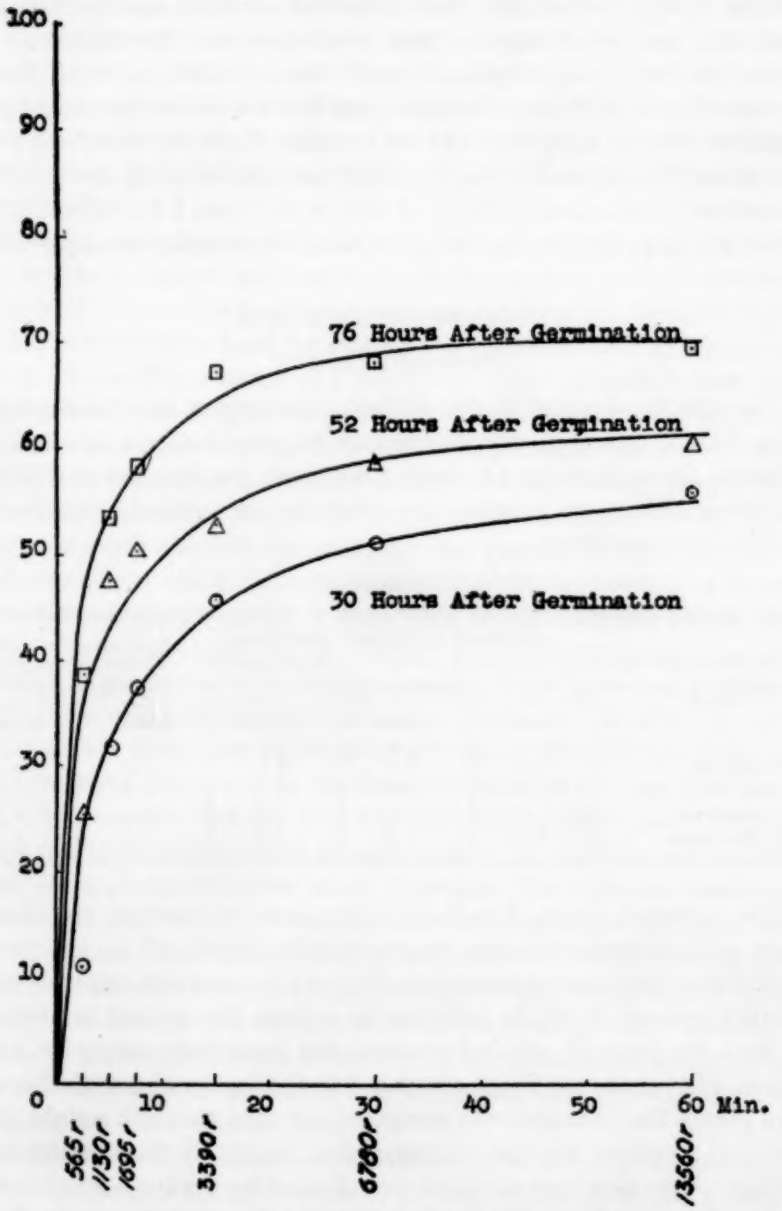

Fig. 2. Percentage reduction of fresh weights of seedlings for the different intervals of measurement. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

vestigated were, with one exception, all inhibitory. Growth, as measured by several criteria, and respiration were checked after irradiation of the seedlings. The one exception noted was in a transitory acceleration of respiration in seedlings which had received a light dosage of irradiation.

Throughout the discussion which follows, it should be borne in mind that the statements made and the conclusions reached are applicable only for the range of x-ray exposures investigated in these experiments, and that the effects observed are described with reference to their manifestation in the responses of the very young wheat seedlings employed in this study.

Influence of x-rays on weights of seedlings

Upon examining the curves of figure 2, it is obvious that in all cases a retardation of fresh weight was brought about by the radiation. The retardation becomes progressively greater as the dosage of x-rays is increased. Retardation, however, is not proportional to the amount of radiation received by the seedlings. Light dosages have a very marked effect. As the dosage is increased, the curve in each case rises more and more gradually, so that it tends to become horizontal. There is little further retardation when the dosage is increased beyond 3390 roentgens (15 minutes' exposure). The general form of the curve is the same for the seedlings that were weighed at an interval thirty hours after germination (five to six hours after the administration of x-rays) as for those weighed seventy-six hours after germination (fifty-two hours after irradiation). The curve for growth retardation, however, rises more rapidly and to a greater height with an increase in the time before measurements are made. Growth retardation therefore becomes more pronounced with lapse of time.

Figure 3 indicates the manner in which the dry weights of seedlings are influenced by longer and, therefore, larger doses of x-rays. As might be expected, the curves of figure 3 bear a close resemblance to those of figure 2. The application of x-rays caused a marked retardation in the accumulation of dry material in the growing parts of the irradiated seedlings. With increase in dosage the effect, like that on fresh weight, increases rapidly at first and then more and more slowly; beyond about 3390 roentgens the effect remains nearly constant. The three curves, representing determinations made thirty, fifty-two, and seventy-six hours after germination, are characterized by a general similarity in shape. They resemble in form those for fresh weights and, like the latter, illustrate the fact that the effect of radiation becomes more pronounced as the seedlings grow older.

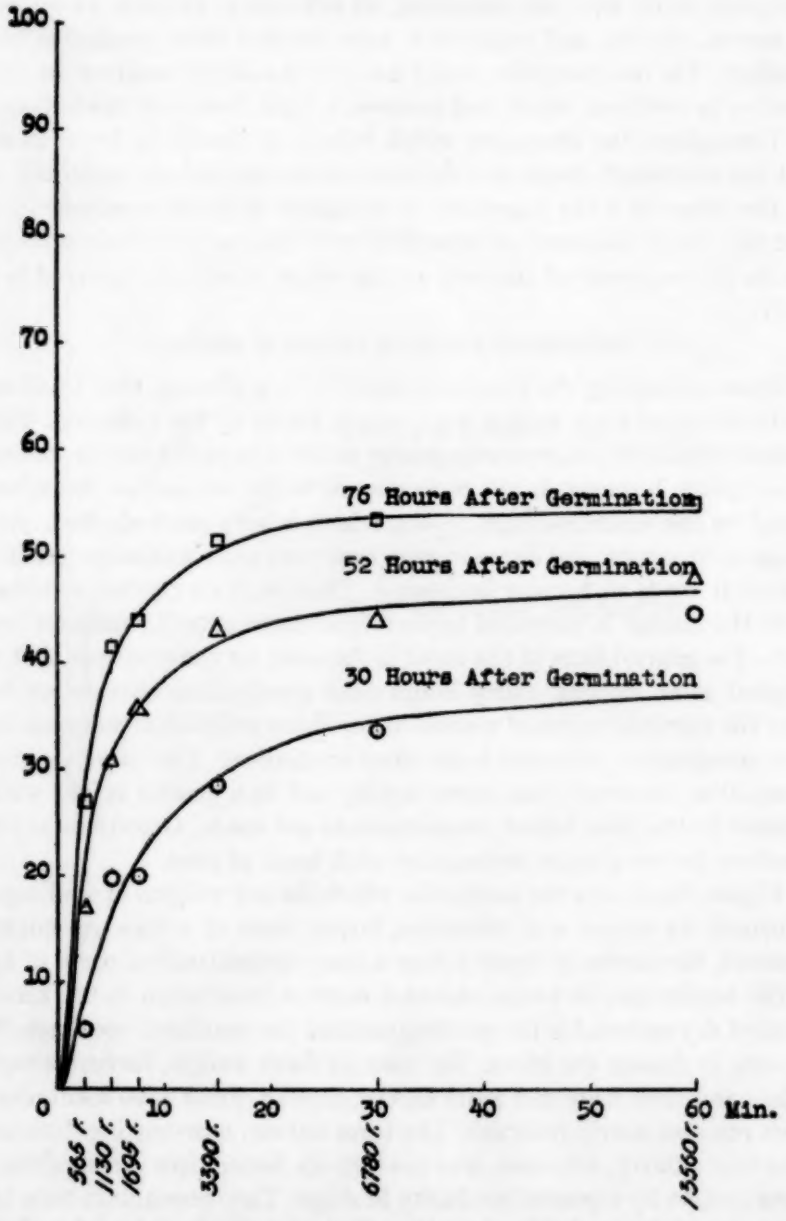

Fig. 3. Percentage reduction of dry weights of seedlings for the different intervals of measurement. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

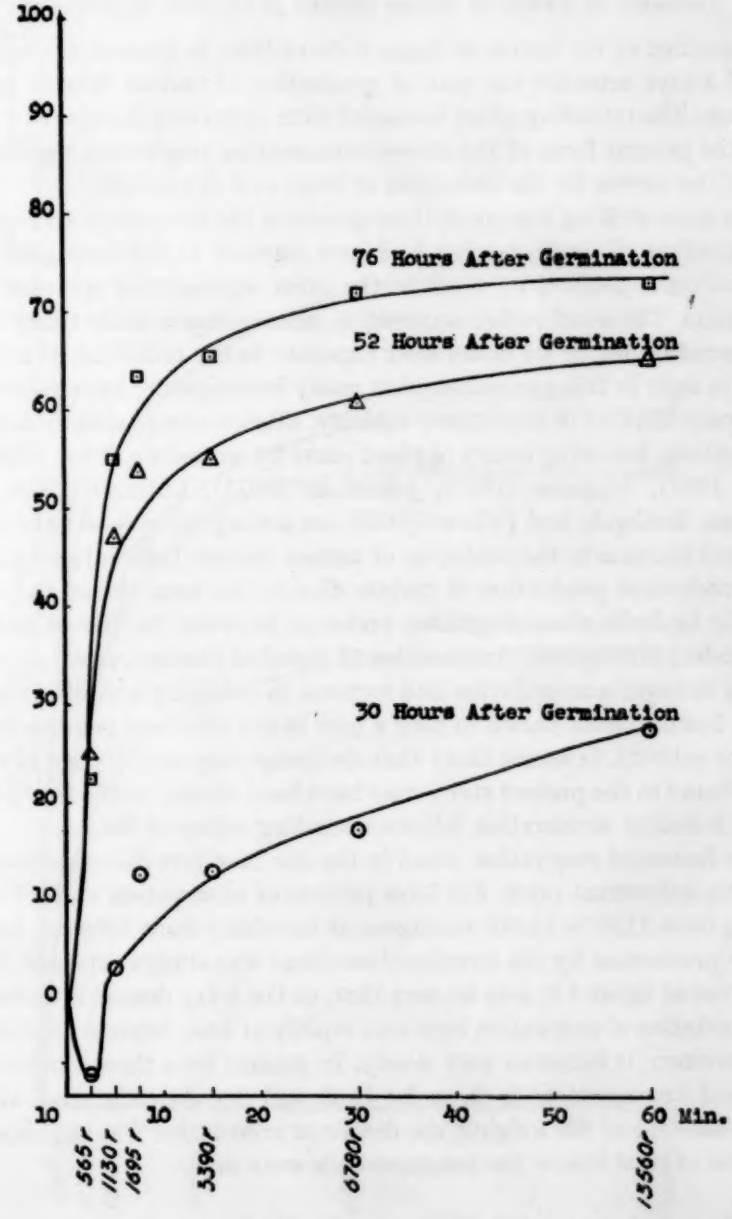

Fig. 4. Percentage reduction in carbon dioxide production of seedlings for the different intervals of measurement. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

Influence of x-rays on carbon dioxide production of seedlings

Inspection of the curves of figure 4 shows that, in general, the application of x-rays retarded the rate of production of carbon dioxide by the seedlings. The retarding effect increased with increasing dosages of x-rays. Thus the general form of the curves representing respiration agrees with that of the curves for the reduction of fresh and dry weight.

The most striking feature of these graphs is the temporary acceleration in respiration of seedlings that had been exposed to 565 roentgens. This acceleration is denoted by a fall in the curve representing retardation of respiration. The acceleration occurred in measurements made thirty hours after germination, or six hours after exposure to the radiation. It is interesting to note in this connection that many investigators have reported a temporary increase in respiratory activity, often accompanied by a rise in temperature, following injury of plant parts by wounding. Thus Richards (1896, 1897), Magness (1920), Johnstone (1925), Lutman (1926), and Coleman, Rothgeb, and Fellows (1928) are among those who have found a marked increase in the evolution of carbon dioxide from injured tissues. This accelerated production of carbon dioxide has been shown to be due partially to facilitation of gaseous exchange between the tissues and the surrounding atmosphere. Acceleration of chemical changes, especially those leading to sugar accumulation and increase in oxidizing and diastatic enzymes, has also been shown to play a part in the observed increase in respiratory activity. It seems likely that the temporary acceleration of respiration found in the present study may have been related to the many cases in which similar acceleration follows wounding injury of tissues.

The increased respiration noted in the one case just discussed was followed by subnormal rates. For later periods of observation and for doses ranging from 1130 to 13,560 roentgens at the thirty-hour interval, carbon dioxide production by the irradiated seedlings was always retarded. From the curves of figure 4 it may be seen that, as the x-ray dosage is increased, the retardation of respiration increases rapidly at first; beyond 3390 roentgens, however, it increases very slowly. In general form these curves show a marked agreement with those for fresh and dry weights. Also, as was true in the case of the weights, the degree of retardation was enhanced by the lapse of time before the measurements were made.

Influence of x-rays on the linear growth of the four organs of seedlings

General influence. The four curves of figure 5 indicate the way in which growth retardation of the four organs of the wheat seedlings is related to increasing doses of x-rays. These curves are based upon measurements of

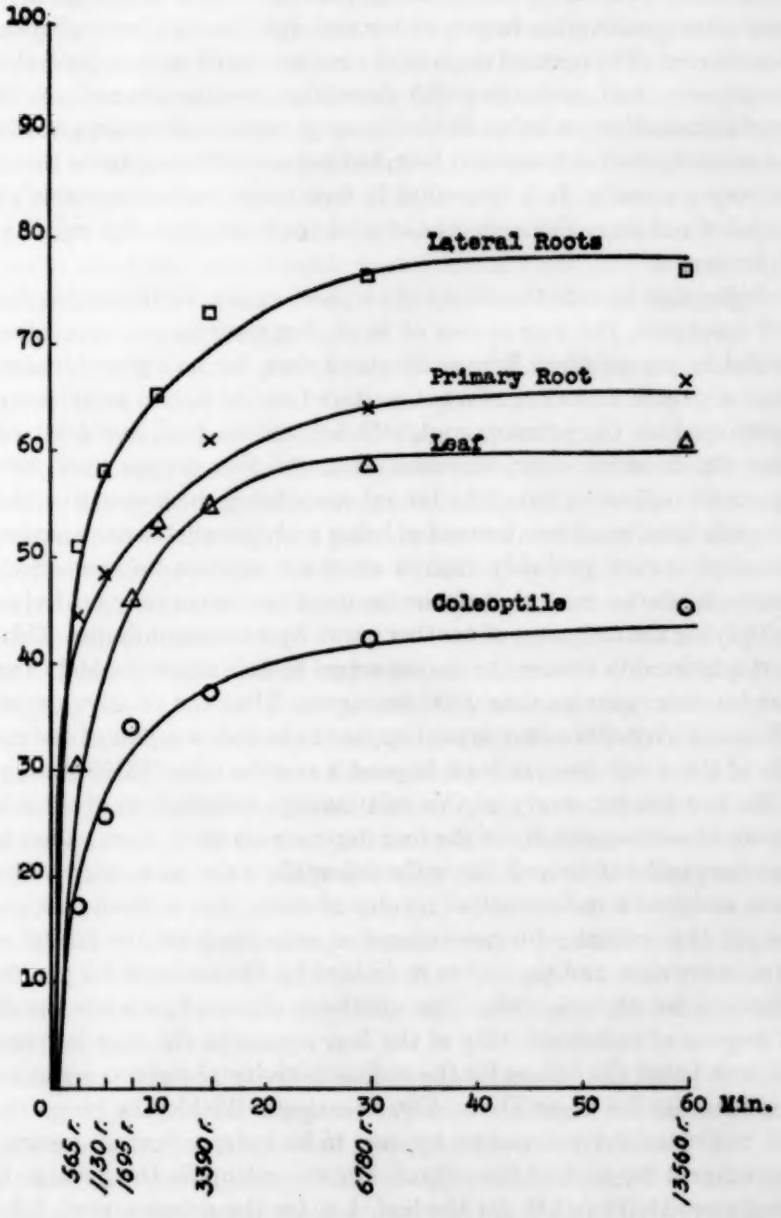

Fig. 5. Percentage reduction in linear growth of the four organs of the seedlings when measured 52 hours after germination began. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

the lengths attained by the growing portions of the seedlings fifty-two hours after germination began, or twenty-eight hours after radiation was administered. The upward slope of the curves for all organs (lateral roots, primary root, leaf, and coleoptile) shows that greater doses of x-rays produced increased retardation in the linear growth of these organs. The increase in retardation is rapid at first; but beyond 3390 roentgens the curves rise very gradually. It is clear that in form these curves resemble closely those derived from the measurements of fresh weights, dry weights, and respiration.

Differences in radiosensitivity of the four organs. In the region beyond 3390 roentgens, the four curves of figure 5 appear to run approximately parallel to one another. It may be noted that, for any given dosage, the greatest growth-retarding effect is produced on the lateral roots; less effect is produced on the primary root, still less on the leaf, and least on the coleoptile. It seems clear, therefore, that the four organs have different degrees of radiosensitivity, the lateral roots being most sensitive and the coleoptile least sensitive. Instead of being truly parallel to one another, the smoothed curves probably bear a constant relation to one another—namely, a relation such that the ordinates of one curve may be derived by multiplying the ordinates of another curve by a constant factor. This constant relationship among the curves seems to hold approximately true, at least for doses greater than 3390 roentgens. Thus the relative degrees of radiosensitivity of the four organs appear to be independent of the magnitude of the x-ray dose, at least beyond a certain value (3390 roentgens).

To test the constancy of this relationship, calculations of the relative degrees of radiosensitivity of the four organs were made from values taken from the graphs of figure 5. Since the coleoptile is the least sensitive organ, it was assigned a radiosensitivity value of unity. For a given abscissa (or dosage), the ordinates (or percentages of reduction) for the lateral roots, the primary root, and the leaf were divided by the ordinate (or percentage reduction) for the coleoptile. The quotients obtained indicate the different degrees of radiosensitivity of the four organs to the dose in question. It is found that the values for the radiosensitivity of a given organ are almost constant for doses above 3390 roentgens. Within the range considered, radiosensitivity therefore appears to be independent of dosage. But it is different for each of the organs. For the coleoptile the average index of radiosensitivity is 1.0; for the leaf, 1.4; for the primary root, 1.5; and for the lateral roots, 1.8.

Effect of time of observation on radiosensitivity. It should be noted at this point that the values for radiosensitivity discussed so far were based upon measurements of the different organs fifty-two hours after germination be-

gan. It is important now to consider what influence the time of measurement may have on these values. Information concerning this question may be obtained by comparing the data based upon measurements of linear growth that were made thirty hours, fifty-two hours, and seventy-six hours after germination began. Since all seedlings were irradiated twenty-four hours after germination commenced, the respective intervals between the time of irradiation and the time of measurement were six, twenty-eight, and fifty-two hours. In considering these results, it should be borne in mind that considerable experimental error may be present in the data secured thirty hours after germination started, owing to the shortness of the organs measured.

Curves plotted to show growth retardation for the thirty-hour and the seventy-six-hour periods exhibit the same general characteristics as those of figure 5, which already have been discussed in detail. Certain differences, however, appear when the curves for these three intervals are compared. The coleoptile and leaf curves practically coincide for the thirty-hour interval, while the leaf and primary-root curves practically coincide for the seventy-six-hour interval. It is difficult to say definitely whether the differences in the three sets of data are really significant or whether they result from experimental error.

The coincidence of the leaf and coleoptile curves for the thirty-hour interval may have resulted from the difficulty of measuring these organs accurately when they were very short and barely, if at all, distinguishable. Although clear differences in radiosensitivity between the leaf and the coleoptile were apparent fifty-two and seventy-six hours after germination commenced, it seems likely that failure to note them at the end of the thirty-hour period was due to the difficulty just mentioned. Furthermore, the coincidence of the leaf and the primary-root curves after seventy-six hours might be attributable to experimental error. By reference to figure 5 and table 1, it will be seen that fifty-two hours after germination the radiosensitivity of the leaf was not very different from that of the primary root (1.4 and 1.5 respectively).

Average values for the relative degrees of radiosensitivity of the four organs for each of the three observation periods are summarized in table 1. The corresponding averages for the fifty-two-hour interval and the seventy-six-hour period agree fairly closely with one another, but they differ from those for the thirty-hour interval. In view of the large experimental error involved in measurements made at the thirty-hour interval, however, the differences which are recorded should probably not be regarded as significant. Accordingly, it seems safe to conclude that the relative degrees of radiosensitivity of the four organs are essentially independ-

ent of the time of observation and the magnitude of the dose administered, within the limits of the experimental conditions involved.

Effect of dose on radiosensitivity. It has been shown in the preceding sections of this paper that the relative degrees of radiosensitivity of the four organs appear to be independent of the magnitude of the x-ray dose administered—within the range of dosages investigated in the present study. As previously pointed out, this generalization appears to hold definitely for the higher portion of the dosage range tested—namely, 3390 to 13,560 roentgens.

It is of special interest to inquire whether this relation exists, even approximately, within a very low range of x-ray dosage. Information bearing on this question is available in a paper by Failla and Henshaw (1931), which reports results obtained with doses ranging from 0 to 1400 roentgens. Figure 6 shows these data plotted in the same manner as the results secured in the present study. A comparison of figure 6 with figure 5 reveals certain differences in the forms and positions of the curves for the corresponding organs. In particular, the curves for the primary and the lateral roots of figure 6 cross twice within the range of 0 to 1400 roentgens, while the curves for these organs do not cross in figure 5; in fact, they remain a considerable distance apart throughout the higher range of x-ray dosages. It is evident also that with the smaller doses represented in figure 6 (0 to 1400 roentgens), there is no constant relationship among the curves for the four organs, such as appears in these curves in figure 5. In the lower range of x-ray dosages, therefore, the relative degree of radiosensitivity of the four organs varies with the magnitude of the dosage.

Effect of temperature on radiosensitivity. In the experiments of Failla and Henshaw (1931) the seedlings were grown at a temperature of 19.5°C., whereas, in experiments described in this paper, the temperature was maintained at 26°C. In order to ascertain whether the differences between the two sets of curves (figure 6 and figure 5) might be due to differences in temperature, several experiments were performed at 19.5°C. The seedlings were exposed to the same doses of x-rays as those employed in the growth tests at 26°C.—namely, 565 to 13,560 roentgens. It is worthy of note that measurements made seventy-two hours after germination began (figure 7) on the seedlings grown at 19.5°C. yielded similar results to those obtained from seedlings maintained at 26°C. Likewise, data obtained ninety-four hours after germination commenced resulted in analogous curves. It seems probable, therefore, that the differences between the results described in this paper and those reported by Failla and Henshaw are not caused by differences in temperature.

The measurements obtained at 26°C. for the seventy-six-hour interval

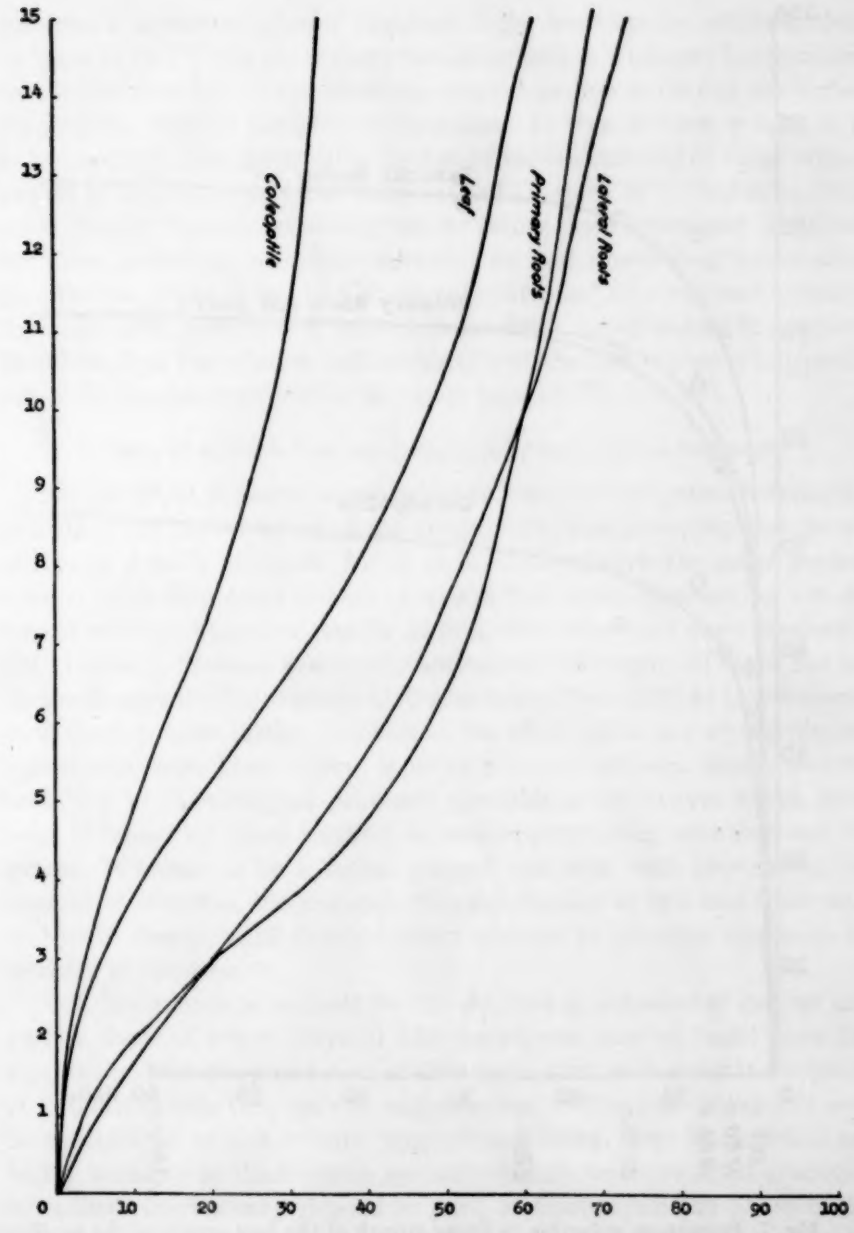

Fig. 6. Percentage reduction in linear growth of the four organs of the seedlings when measured 94 hours after germination began (taken from the data of Failla and Henshaw, 1931). The ordinates represent dosage in roentgens × 100; the abscissas represent percentage reduction.

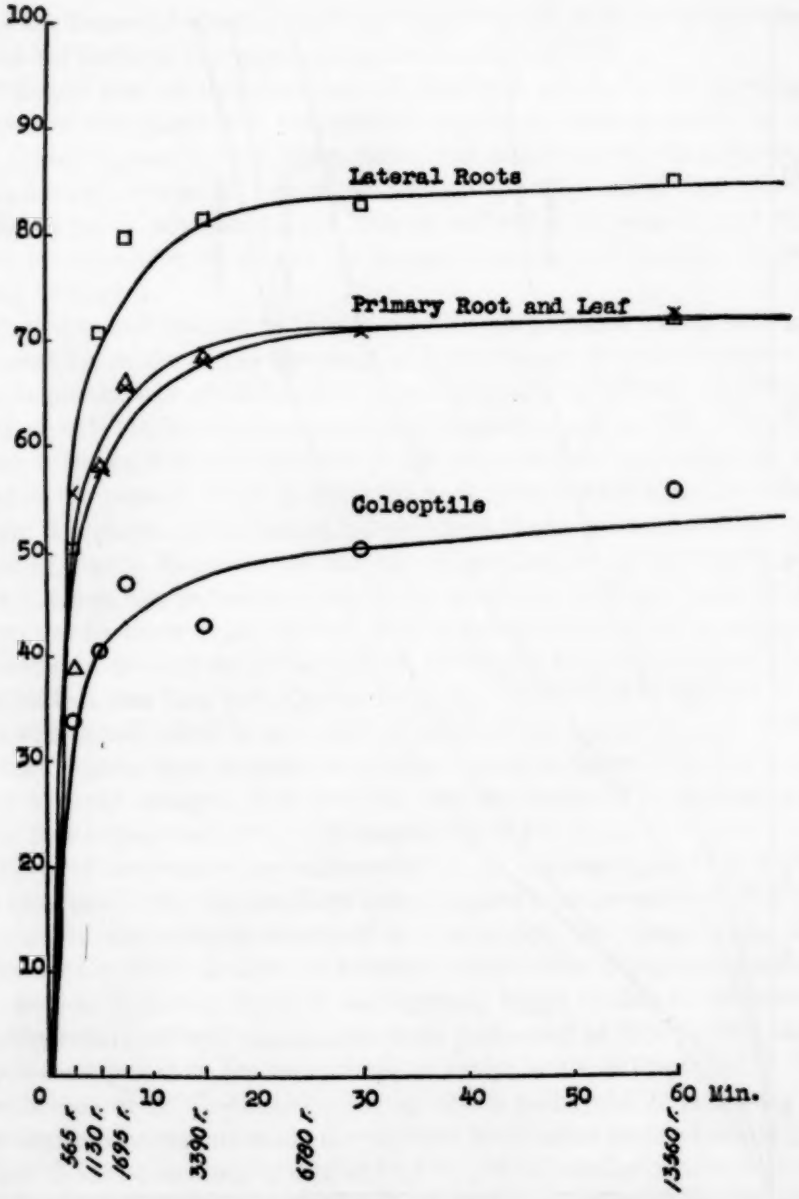

Fig. 7. Percentage reduction in linear growth of the four organs of the seedlings when measured 72 hours after germination began, when the temperature was varied to 19.5°C. The ordinates represent percentage reduction; the abscissas represent dosage —the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

manifest a somewhat greater response of the seedlings to radiation than do those at 19.5°C. for the seventy-two-hour period. This may be explained by the fact that the control seedlings attain a greater growth at the higher temperature than at the lower temperature. In view of these results, it is to be expected that the relative degrees of radiosensitivity of these organs should be approximately the same at 19.5°C. as at 26°C. (table 1). And these degrees of radiosensitivity are in rather close agreement for all of the time periods in question—seventy-two and ninety-four hours after germination began in the 19.5°C. experiments, and fifty-two and seventy-six hours after germination started in the 26°C. experiments. It appears, therefore, that the relative radiosensitivity of the four organs is independent of the temperature within the range from 19.5°C. to 26°C.

General relation between dosage and physiological response

At this point it seems worth while to consider the general similarity in form of the curves for all of the different physiological processes shown in figures 2 to 5, inclusive. All of these curves follow the same general course. With increasing dosage of x-rays the curve representing the response in every case rises rapidly at first, then more and more gradually, until it finally becomes practically horizontal. The region of rapid rise includes dosages from 0 to about 3390 roentgens. From 3390 to 13,560 roentgens there is little further increase in the effect upon any of the physiological processes. These curves showing relations between dosage and retardation of physiological processes resemble many curves which have been obtained by other workers in studies correlating stimulus and response. It seems to be a rather general rule that with increase in the amount of stimulus, the response increases rapidly at first and then more and more slowly, until finally further increase in stimulus results in no increase in response.

One hypothesis to account for the decreasing influence of greater and greater doses of x-rays (beyond 3390 roentgens) may be based upon the supposition that the population of cells making up each organ is composed of individual cells that vary in radiosensitivity. The individual cells may be assumed to exhibit a wide range of sensitivity, from those which are highly sensitive to those which are only slightly sensitive. This gradation in radiosensitivity may depend in part, at least, upon the phase of development of the cells; rapidly dividing cells probably are most susceptible to radiation (law of Bergonié and Tribondeau, 1906). The most sensitive cells would be influenced by the small doses of x-rays, but the most resistant ones would not be affected appreciably even by the largest doses employed in these experiments. According to this view, the rapid initial rise

of the curves of figures 2 to 5 indicates that most of the cells in each of these populations are markedly affected by doses smaller than 2260 or 3390 roentgens. The other cells are not markedly affected by doses four times as great. In all cases some growth takes place, even with the largest doses employed. Great emphasis should be placed upon the fact that it is difficult to bring about complete cessation of growth and respiration by means of x-ray treatment. This is in marked contrast to the ease with which all vital activity of seedlings may be inhibited subsequent to the application of chemical poisons.

It would be of interest in this connection to correlate growth retardation with anatomical and cytological features of the plant organs in question. Detailed observations, however, are not available at present for an interpretation along these lines. Rapid cell division in the root is largely confined to a very short apical region, and most of the elongation of the root occurs in a short zone behind the region of cell division. According to Tetley and Priestley (1927), cell division in the coleoptile ceases at a very early stage. They state that if the coleoptile of a wheat grain that has been soaked in water for a few hours is removed and sectioned, all the regions of the coleoptile except the vascular strands will be found to be vacuolating. These investigators were not able to detect cell divisions in the tissues of the coleoptile. Growth of this organ depends almost entirely upon the elongation of cells in a zone lying a short distance below the apex. The fact that cell division in the coleoptile ceases at such an early age may account for its low radiosensitivity. Growth of the leaf is brought about largely by cell division and enlargement in a zone near the base of the leaf.

It is reasonable to expect that a retardation in the accumulation of fresh and dry materials and in the production of carbon dioxide would accompany a retardation in the rate of linear growth of the organisms. Therefore, the similarity of the effects of x-rays on the different processes investigated would be expected.

Correlations between different responses to radiation

Certain interesting relations between different responses to radiation may be brought out by determining the ratios existing between corresponding sets of data for any two physiological processes. For example, the amount of carbon dioxide produced by seedlings which had received a given dose of x-rays may be divided by the value of the fresh weight for seedlings exposed to the same dose. Similar ratios may be secured for each interval of measurement. From this procedure it is possible to ascertain the exact quantity of carbon dioxide liberated by a given set of seedlings per gram of fresh weight per hour.

The experimental data for the other physiological processes investigated were correlated in a manner similar to that just described. A summary of the results is presented in table 2. In this table one column contains the values obtained for non-irradiated seedlings, while the other column includes the averages of ratios determined for irradiated seedlings.

A supplementary means of correlating the experimental results is afforded by epitomizing the data in tabular form, as in table 3. In this table

TABLE 2

Summary of correlations of experimental data

RATIO	MEASURING INTERVAL (hours)	CONTROL SEEDLINGS	IRRADIATED SEEDLINGS
Mg. CO_2 per gram fresh weight per hour	30	3.01	4.51
	52	1.99	1.90
	76	1.04	1.03
Mg. CO_2 per gram dry weight per hour	30	30.6	36.7
	52	19.6	14.9
	76	13.0	9.5
Mg. fresh weight per mm. average linear growth	30	12.6	10.9
	52	116.5	131.2
	76	132.8	178.7
Mg. dry weight per mm. average linear growth	30	12.4	13.2
	52	11.8	16.7
	76	10.7	19.7
Mg. fresh weight per mg. dry weight	30	10.1	8.2
	52	9.9	7.9
	76	12.5	9.2
Mg. CO_2 per mm. average linear growth per hour	30	.38	.49
	52	.23	.25
	76	.14	.18

the percentage reduction obtained for each exposure is presented for each method of measuring the effects of radiation on the wheat seedlings and for all intervals of measurement. From the data thus arranged, a direct comparison may be made between any two responses of the seedlings to x-rays.

Carbon dioxide production and weights. Inspection of table 3 shows that for all exposures when measurements are made thirty hours after germination began, the amount of fresh weight is reduced to a greater extent than is the carbon dioxide production. Reference to table 2 shows that at the thirty-hour interval the rate at which carbon dioxide is produced by the irradiated seedlings is more rapid than that for the non-irradiated seed-

lings of the same fresh weight. It is evident, therefore, that the effect of radiation is to accelerate the production of carbon dioxide per unit of fresh weight at this period.

It will be seen that for periods longer than thirty hours after germination, the total fresh weight is reduced approximately to the same extent as is the amount of carbon dioxide production, following irradiation. From these considerations it appears that while x-rays retard the growth of the

TABLE 3

Percentages of reduction obtained at different intervals for various methods of measurement

MINUTES' EXPOSURE	FRESH WEIGHT	DRY WEIGHT	CARBON DIOXIDE PRODUCTION	LINEAR GROWTH
30 hours after germination				
2.5	11.1	5.6	−7.7	15.7
5.0	31.8	19.8	3.1	22.8
7.5	37.5	20.0	12.6	28.9
15.0	45.7	28.7	12.9	30.0
30.0	51.1	33.7	17.2	36.9
60.0	55.7	44.7	27.4	44.1
52 hours after germination				
2.5	25.4	17.1	25.2	39.9
5.0	47.7	30.9	47.3	47.3
7.5	50.3	35.7	53.7	53.4
15.0	52.8	43.2	55.2	59.7
30.0	58.6	44.3	60.8	63.4
60.0	60.2	48.2	65.3	65.3
76 hours after germination				
2.5	38.6	27.1	22.5	48.5
5.0	53.5	41.4	55.0	65.6
7.5	58.2	44.0	63.2	70.6
15.0	67.2	51.5	65.4	74.8
30.0	67.9	53.4	71.9	76.4
60.0	69.2	54.6	73.2	78.7

seedlings in mass, they bring about an equivalent reduction in the production of carbon dioxide. Thus, the metabolic activity of the irradiated organs, mass for mass, remains essentially the same as that for the non-irradiated organs, at the later periods of measurement, and for the amount of radiation administered in this investigation.

If, on the other hand, dry weights are correlated with the carbon dioxide production, there is a greater reduction in carbon dioxide production than in the total dry weight, except thirty hours after germination, when the reverse is true. Thus, at the later intervals of measurement less carbon

dioxide is produced by the irradiated seedlings than by the non-irradiated seedlings of a given dry weight. It is evident, therefore, that one effect of x-rays in the doses administered is to depress the carbon dioxide production per unit of dry weight, except thirty hours after germination, when the effect is to stimulate the respiration per unit of dry weight.

Weight and length. Since it was impossible to weigh the various organs separately, the correlation of weights and lengths has been made on the basis of the average lengths of all four organs (coleoptile, leaf, primary and lateral roots). Comparison of the two sets of data makes it evident that, for a given exposure, there is, in general, a greater percentage reduction in linear growth than in the amount of fresh weight of the irradiated seedlings, except thirty hours after germination. Thus, for the same fresh weight, the average length of the four organs is less in the irradiated seedlings than in the controls. In other words, the irradiated organs are somewhat heavier per average unit length than the non-irradiated organs. These relations between weight and length do not apply to seedlings measured five to six hours after the administration of x-rays, or thirty hours after the beginning of germination. At this stage the fresh weight seems to be more retarded than the linear growth of the irradiated seedlings. This effect probably is not very significant, owing to the shortness of the organs and the correspondingly large experimental error involved in measurements made at this period.

When dry weights are correlated with the average lengths of the organs, it is evident that there is less reduction in the dry weights than in the average linear growth of the irradiated seedlings. Accordingly, an effect of x-rays in the doses administered is a stunting of linear growth, accompanied by a slight increase in the average diameter of the organs. The differences in diameter were noticeable during the course of the experiments, although they were not measured.

Water content. When the dry weights of seedlings are correlated with the fresh weights, it is found that the irradiated seedlings have lower fresh weights than the non-irradiated seedlings of the same dry weight. In other words, there is a greater percentage reduction in fresh weight than in dry weight in the irradiated seedlings. Hence, the irradiated seedlings contain less water than the non-irradiated seedlings of the same fresh weight. This statement is true for all intervals of measurement and for all doses of x-rays administered.

The effect of x-rays in producing a relative reduction in water content of the organs of the wheat seedlings is probably due to decreased imbibitional or osmotic powers of the irradiated organs.

Linear growth and carbon dioxide production. If the carbon dioxide pro-

duction of the seedlings is correlated with the average linear growth of the four organs, it is apparent that for a given reduction in carbon dioxide production, there is, in general, a greater reduction in the average linear growth of the irradiated organs. This is not so obvious at the fifty-two-hour interval as at the other two periods of measurement. Generally speaking, however, the irradiated seedlings respire at a more rapid rate than the non-irradiated seedlings of the same average length. Thus, x-rays, in the doses administered, increase the rate of respiration per unit length of the organs.

General correlations between physiological responses to x-rays. When a general survey is made of table 3, two facts of interest become evident. In the first place, the percentage reduction obtained in a particular response to a given dose of x-rays is greater with an increasing interval before measurement of the effect. For example, at the age of thirty hours the percentage reduction in linear growth of the seedlings which received sixty minutes of treatment is 44; at the age of fifty-two hours it is 65; and at seventy-six hours, 79. It is clear, therefore, that there is a latent period in the biological action of radiation, whereby the observable effect becomes more marked with the lapse of time between irradiation and measurement of the response. It is impossible to say that the maximum effect was obtained with the time intervals used in the present experiments. If observations had been made at longer intervals, it is probable that still more marked effects would have been found.

In the second place, there is a close correlation among the percentages of reduction obtained for fresh weights, carbon dioxide production, and linear growth, as measured at the later periods. From this fact it appears that any one of these effects might be taken as a criterion of general injury to the organism, for certain practical purposes.

Apparent correlation between radiation effects and aging. Several investigators have obtained evidence which indicates that the application of x-rays causes modifications of the irradiated material that are associated with senescence. Isaacs (1932) was of the opinion that x-rays speed up the life processes in the blood cells, thus hastening the advent of old age. Nemenow (1925), citing the results of a number of investigators who had studied the action of x-rays on living materials, called attention to the fact that the application of x-rays brought about changes which were characteristic of those accompanying old age.

In view of these observations, it seemed of interest to analyze the data obtained from the present experiments to see whether a similar conclusion might be drawn from them. The values presented in table 2 serve as a basis for this analysis. Thus, at the thirty-hour age the amount of carbon di-

oxide liberated per hour per gram of fresh weight for the non-irradiated seedlings is 3.01 milligrams. At the fifty-two-hour age the corresponding index for the non-irradiated seedlings is 1.99 milligrams per hour per gram. Similarly, at the seventy-six-hour age the respiratory index has a value of 1.04 milligrams per hour per gram. Hence, with increasing age, the rate of respiration per gram of fresh weight decreases in the control seedlings.

If the irradiated seedlings had been so modified as to manifest characteristics associated with senescence, it follows that the respiratory index should fall below that which is characteristic of the non-irradiated seedlings of the same age. It is demonstrated clearly in table 2 that there is no significant difference between the values of the respiratory index for the irradiated seedlings and those for the controls in the case of measurements made at the later intervals after germination began. On the contrary, the respiratory index of the irradiated seedlings which were measured at the thirty-hour interval is higher than the index for the non-irradiated seedlings of the same age. Hence, five or six hours after irradiation the x-rays have affected the seedlings in such a manner as to change the index to that characteristic of non-irradiated seedlings of younger age. Accordingly, it may be concluded that the effect of x-rays on the growing parts of the wheat seedlings is a stimulation of respiratory activity, followed later by a return to normal activity. The evidence obtained from the respiratory index, then, does not indicate the advent of premature senescence in the irradiated seedlings. It suggests, rather, a temporary setback, followed by the resumption of behavior characteristic of normal seedlings of the same age.

The remaining experimental data have been considered with reference to their significance in demonstrating premature senescence in irradiated seedlings. Correlations of carbon dioxide production with dry weight at the later measuring intervals suggest premature aging in the irradiated seedlings. Likewise, the values obtained for the ratios of fresh weight to linear growth at the later intervals are characteristic of senescence in irradiated seedlings. In general, however, the irradiated seedlings appear to be stunted, rather than advanced in age. Thus, the evidence obtained from the present investigation offers no conclusive corroboration of the theory that x-rays induce premature senescence.

SUMMARY

This paper presents the results of an experimental study of the influence of x-rays on the growth and respiration of very young wheat seedlings. After being soaked in water for three hours, the seeds were planted on moist filter paper and left to germinate. At the end of twenty-four

hours the seedlings were irradiated with doses of x-rays ranging from 565 to 13,560 roentgens. Periodic determinations were then made of respiratory activity and growth during the first hundred hours of seedling development. The main results of this study are given below. The conclusions drawn from these experiments can be regarded as applicable only for the range of x-ray exposures used, the time intervals involved, and the particular biological material investigated in this work.

1. The effects of x-rays on wheat seedlings were found to depend upon (*a*) the dose of radiation administered, (*b*) the time when the effect is observed, and (*c*) the type of response considered.

2. Retardation of both fresh-weight and dry-weight production by the growing parts of the seedlings was brought about by all doses of x-rays employed, and this retardation became progressively greater with lapse of time and with increase in dosage.

3. Respiration was depressed in all cases except those in which seedlings were tested five or six hours after irradiation (i.e., thirty hours after the beginning of germination). At that time the seedlings which had received the smallest dose of radiation (565 roentgens) exhibited an accelerated rate of respiration.

4. Retardation of linear growth of the coleoptile, the leaf, the primary root, and the lateral roots was obtained with all doses and time intervals employed.

5. Each of the organs investigated seemed to have a characteristic degree of radiosensitivity. The lateral roots were most sensitive and the coleoptile was least sensitive. The relative degrees of radiosensitivity of the four organs studied appeared to be essentially independent of time of observation, of dosage, and of temperature—within the particular ranges of these conditions investigated.

6. Certain correlations between different responses to radiation were noted, as follows:

(*a*) X-rays did not affect the rate of carbon dioxide production per unit of fresh weight when the seedlings were more than fifty-two hours old. They increased the value of this quotient thirty hours after germination (five or six hours after irradiation).

(*b*) The rate of carbon dioxide production per unit of dry weight was lower in the irradiated seedlings than in the non-irradiated seedlings, fifty-two or more hours after germination began. This ratio was greater in the irradiated seedlings thirty hours after germination.

(*c*) Stunting of linear growth was accompanied by a reduction in water content and a slight increase in weight per unit length of the organs in irradiated seedlings.

(*d*) The irradiated organs respired at a more rapid rate than the non-irradiated ones of the same average length.

In all these cases the effect was more apparent, the larger the dose and the longer the interval before measurements were made.

7. The data presented were analyzed to determine their bearing on the problem of aging as manifested in the experimental material. In view of the conflicting evidence obtained from these data, it was suggested that the theory that irradiation induces premature aging was not corroborated by the present experiments.

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The relation of Uromyces Caladii and other rusts to their hosts

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(WITH PLATES 6–8)

Dufrenoy (1928, 1929) in a series of articles upon relations between seventeen plant parasites and their hosts, reaches conclusions as to the effect of the haustoria on the host cell quite the opposite to those which I have stated in connection with my own observations upon the rust parasites. The following haustorial parasites were studied by Dufrenoy: *Puccinia Sorghi, Puccinia Asphodeli, Uromyces Caladii, Peronospora Schleidenii, Phytophthora infestans, P. terrestris, Blepharospora cambiovora* and *Fusarium Rubi*. Later (1930), he adds observations upon *Helminthosporium* in barley and *Pseudoperonospora humuli*.

Dufrenoy (1928) thinks that the finely vacuolate condition of the protoplasm which is, at times, found in healthy cells in periods of metabolic activity is brought about by a return from the univacuolate condition of more mature cells to the more foamy condition of embryonic cells and he interprets this as a fragmentation of the vacuolar system effecting an increase of contact surfaces between cytoplasm and vacuoles. Dufrenoy finds in cells infected by fungous parasites, instead of the normal central vacuole, a system of small vacuoles lying in a network of cytoplasmic strands. He thinks this condition is the same in origin and in function as that described for non-infected cells under conditions of metabolic activity. Even more important than this fragmentation of the vacuole he considers the physico-chemical modifications wherein various substances held in solution in the normal vacuole are precipitated as phenolytic compounds in the infected cell. "Plus importantes encore, sans doute, que les modifications morphologiques des vacuome, sont les modifications physico-chimiques de son contenu." (1928).

Dufrenoy's observations of the phenomena of infection are most specifically described in his studies of haustorial parasites. He states (1929) that in the case of *Uromyces Caladii* the entrance of the haustorium causes a condition in the host cell comparable to that described by Magrou (1918) as phagocytosis when orchid cells digest their mycorrhizal invaders. With respect to *Pseudoperonospora humuli* Dufrenoy (1930) compares the condition which follows haustorial entrance to that observed in cells of carnivorous plants by Magenot: "une protéolyse locale, et une fragmentation de l'appareil vacuolaire en un système de petites vacuolaires à contenu riche en protides." He further holds that the condition is advantageous to the haustorial parasite in that it involves an increase of the biochemical reactions and an exaggeration of the processes of disintegration of the al-

bumenoid complexes. "Le suçoir coiffé d'un cytoplasme finement vacuolisé, plonge donc dans un mileu riche en protides solubles."

I have myself found little indication of changes in the host-cell protoplasm around haustoria, but it is entirely possible that an irritation set up by a haustorial invasion might lead to an at least apparent increase of protoplasm in the cell. Such an increase might become visible as a finely vacuolated thickening of the primordial utricle around a haustorium and even over the whole inner surface of the cell. I do not think that this condition need imply the origin of the small vacuoles from a large central vacuole any more than it need imply such an origin for the vacuolization accompanying proteolysis in the uninfected cells to which Dufrenoy refers. Such a vacuolization, instead of being considered a fragmentation of a central vacuole, could be brought into line with the Dangeard and Guilliermond (1929) theory of the origin of vacuoles from small solid "mitochondria-like bodies" by the swelling up and solution of the latter. It may also be noted that precipitation products in vacuoles stained with neutral red are described by Guilliermond as an occurrence in normal cells. He says (1929) that neutral red may thus be considered virtually a specific dye for the vacuome and that as the cell ages it is known that the vacuole shows one of two very different aspects: (1) Numerous thread-like, sticky bodies staining uniformly and deeply with neutral red. (2) Fewer and larger liquid vacuoles in whose paler contents deeply staining precipitates are made to appear through the action of neutral red. Although Dufrenoy found this precipitation only in the vacuoles of infected cells it is to be questioned whether, in view of Guilliermond's work, such a distinction would prove constant.

In all these cases of haustorial parasites Dufrenoy finds that the changes in the vacuoles are preceded and accompanied by plasmolysis of the host cell. "Lorsqu'un filament de rouille pénètre une cellule, la vacuole se rétracte, entrainant la couche du cytoplasme, et la cellule parâit plasmolysée." By cell penetration in this connection Dufrenoy apparently means only the penetration of the cell wall. According to his interpretations of his figures and descriptions of the conditions in *Uromyces Caladii* the haustorium would seem to exert a stimulus upon contact with the plasma membrane which results in excess exosmosis, causing first plasmolysis and then changes in the vacuolar condition. The result is very different, as shown in his figure 1 (1929), from that in my figure 2 of the present article where the contracted cytoplasm is clearly invaginated by a haustorium. In the matter of penetration and of the reaction of the host cell I found that in the corn rust and several other rusts the host cell cytoplasm upon a penetration of a haustorium was invaginated and was not plasmolysed.

The studies of Plowe upon membranes in the plant cell (1930, 1931) give indirect support to the probability of invagination rather than puncturing by haustoria. She emphasizes the elasticity of the plasmalemma by noting that it remains intact about a needle thrust deeply into the protoplasm of an onion cell. Plowe writes: "It is no easy matter to puncture or tear the plasmalemma of the living protoplast. Even a very sharp needle carries a layer of protoplasm with it as it enters the cell: the needle invaginates, rather than pierces the protoplast; the plasmalemma lies next the needle, and the mesoplasm and tonoplast are also indented and carried in."

I found in all the rusts which I have studied that even in cases of extreme and advanced infection there was little change in the appearance of the cytoplasm of the host cells and I concluded (1927), "For the Uredineae in general, I think that an elaborate development of the haustorium has furthered the lack of disturbance in the regions of special metabolism of the host-parasite complex." This same view has recently been supported by Hull (1931) in discussing types of resistance to *Puccinia Sorghi* shown by *Zea Mays*. After describing, for a resistant host, the plasmolysis of both host and parasite cells upon the first formation of primary hyphae, before any haustoria have a chance to form, she notes an exception as follows: "When large substomatal vesicles are formed in the resistant host the fungus is able to proceed with its life-cycle up to the period of sporulation. Growth is more rapid than in the susceptible host and haustorial contacts are made with host cells before the latter have time to establish any antagonistic response. *Once this contact is complete there is a perfect equilibrium between host and fungus and henceforth formation of haustoria produces no noticeable reaction in the host cells.*" (Italics are mine.)

I have read with especial interest Dufrenoy's report upon *Uromyces Caladii* because I also have made a study of the host-parasite relations of this rust. Dufrenoy in his first article (1928) writes that in the infected cells the vacuole fragments in such a way as to surround an haustorium with an aureole of small vacuoles and that most of the vacuoles in the vicinity of haustoria form, with neutral red, precipitations applied to the surface of the vacuoles until there is no color left within the vacuole. Then "La cellule d'ailleurs perd sa turgescence. Le cytoplasme, qui se détache de la paroi cellulaire prend les aspects que les descriptions de Guilliermond ont rendu classiques pour les cellules plasmolysées." (1928)

In his second article (1929) Dufrenoy describes for the same rust, the fragmentation of the vacuome into a group of small vacuoles "autour du sommet du filament de rouille." It is to be noted that in this description

Dufrenoy uses the term, "filament" instead of the term "suçoir" for the intracellular hypha.

When Dufrenoy speaks of a filament of the rust penetrating a cell one may question whether he is using the term *filament* as synomymous with *haustorium*, or whether he is describing intracellular filaments distinct from the haustoria whose action he described in the first article upon *Uromyces Caladii* (1929). Figure 1 printed in his first article indicates that he distinguishes between the two structures since he figures an epidermal cell which contains both a coiled structure labelled haustorium and an elongated hypha labelled filament. The legend of figure 3 reads, "Cellule traversé par un filament de rouille, Le filament a émis un suçoir." However, in the second article he labels as haustorium both a detached branching coil within the cell, and a long hypha which apparently enters from the adjoining cell. The method of penetration of these filaments, whether they are intracellular hyphae or haustoria, is, according to these figures, most unusual. There is no constriction shown at the point of entrance and the cell wall of the host is drawn without break across the full width of the hypha. This may indicate that the point of penetration is at a different plane than that of the figure. Such a condition is figured by Evans (1933) for *Urocystis cepulae* where intracellular hyphae are passing out into intercellular spaces but Evans has also figured hyphae in the penetration plane where there is a well marked aperture in the host cell wall. The constriction of hyphae as they penetrate a wall is found even in the case of such facultative parasites as *Botrytis*, *Rhizopus*, or *Pythium*:—witness the figures of Hawkins and Harvey (1919) for *Pythium debaryanum*. Since it is characteristic of the majority of rust haustoria that penetration is effected by a papilla which leaves no appreciable opening in the host cell wall and which remains as a narrow neck at the base of the haustorium, the manner of penetration of such unconstricted hyphae as Dufrenoy shows needs explanation or comment.

In figure 1 in his second article Dufrenoy figures a haustorium with a narrow neck such as I have found most typical for rust haustoria and such as I have figured for *Uromyces Caladii* in figure 56 (1927) and more strikingly in figures 2 and 6 of the present article. If *Uromyces Caladii* has both intracellular hyphae, and haustoria with differing penetration habits it would seem reasonable to expect that they might affect the host cell differently. Yet Dufrenoy treats them as the same in reaction and, as I have already noted, in his second article calls them both haustoria.

I have never found intracellular hyphae in the case of *Uromyces Caladii*. The only intracellular structures I have found are coiled and lobed haustoria which show a slender basal stalk or neck if the section is cut at the

plane of their penetration. I am convinced that these hyphae which Dufrenoy figures crossing walls of the host cells without constriction are not intracellular but are intercellular hyphae which lie at a different level from that of the cells figured, though it is hard to understand how Dufrenoy could have made such an error of observation.

Uromyces Caladii infects both epidermis and chlorenchyma of *Arisaema* leaves with a vigorous growth of intercellular hyphae and of haustoria. Intercellular hyphae make an especially thick felt in the space between lower epidermis and spongy parenchyma. Here are the anlagen of spermogonia and aecidia, and later, of teleutosori. In cross sections of leaves the intercellular spaces next the lower epidermis are often so enlarged as to seem like cells filled with hyphae. Only the observation of the cell angles, the presence or absence of the primordial utricle, and of plastids, enables one to distinguish between cell cavity and intercellular space. See figure 3, a detail from a cross section of a leaf which bore erumpent spermogonia. Here a mass of hyphae lies in an enlarged intercellular space between lower epidermis and spongy parenchyma.

Dufrenoy, however, made his observations upon tangential views of cells obtained by stripping shreds of epidermis from a leaf. Any teacher who has used strips of onion epidermis for the demonstration of protoplast structure knows the difficulties occasioned by the third dimension in tangential views of epidermis to which a layer of parenchyma often clings.

Since seeing Dufrenoy's figures and descriptions I have made studies of fresh epidermal strips in addition to those of cross sections of embedded material. I mounted the fresh material, as Dufrenoy did, in 8% sugar solution with 10% neutral red for stain. Dufrenoy also used osmic-chromic acid fixation and fuchsin stain but apparently upon similar epidermal strips and not upon embedded material.

I have shown in figure 1 a tangential view of the under epidermis of a leaf which was mounted with the outer side up. A haustorium is seen in the central cell; the curve of its narrowed base indicates that its penetration point is in a lower plane. It probably arises from a branch of the intercellular hypha which shows through the adjoining cell from below and disappears under the cell wall. Another hyphal branch may be seen, through the cell, ending beneath the stoma. The three hyphae whose tips lie apparently within, but in reality below, epidermal cells are similar to those which Dufrenoy calls filaments in his first article and haustoria in his second. The short branch beneath the lower, lefthand cell is quite similar to the branch within a cell which Dufrenoy labels haustorium in figure 3 of his second article. It is apparent that the cell walls and the cytoplasm which lie across or around the hyphae in my figure lie in an upper

plane and have no connection with the hyphae. There was no evidence of plasmolysis in any of these cells. The 8% sugar solution was isotonic for infected as well as uninfected cells instead of for the latter only as Dufrenoy states. Figure 2 shows an epidermal cell drawn from the same mount as that of figure 3, after it had stood for an hour. Evaporation had concentrated the sugar solution and all the cells were plasmolyzed but in this state there was, perhaps, even more conclusive evidence for invagination of the cytoplasm by the haustorium.

Figure 6 shows another epidermal cell which contains a haustorium. The characteristic slender neck of the haustorium may be noted. The haustorium tip lies near the centrally located nucleus. A bundle of raphides lies in the large vacuole. Here again there is evidence of invagination of the host cytoplasm by the haustorium. There is no greater evidence of fragmentation of the vacuolar system by a network of cytoplasmic strands than is often the case with centrally located nuclei in uninfected cells.

Figures 4 and 5 were drawn from a shred of lower epidermis to which patches of spongy parenchyma adhered. It was mounted inner side up. Figure 5 should overlie the right-hand part of figure 4. Figure 4 shows the median optical plane of the epidermal cells; figure 5, the median optical plane of the parenchyma cells. Two of the epidermal cells contain large, coiled haustoria, outgrowths, presumably, of intercellular hyphae which lay just above. Such a hypha is shown in figure 5, issuing from below a parenchyma cell and overlying the epidermal cells. The entire lack of plasmolysis in the infected cells is the more evident because of the tiny plastids which lie in the primordial utricle. These were leucoplasts and were sharply distinguishable in size and color from the chloroplasts of the parenchyma cells. This tissue was mounted in 8% sugar solution but was unstained.

Figures 7 and 8 show lower and upper planes respectively of an epidermal cell which was mounted inner side up. The intercellular hypha plainly overlies the cell; the lower branch of the hypha in figure 8 is similar to the one labelled haustorium in figure 3 in Dufrenoy's second article (1929). Figure 9 gives another view of the inner side of epidermal tissue drawn at the plane of the intercellular hyphae. The cytoplasm around the tip of the hypha might indicate plasmolysis if one considered the hypha to lie in the plane of the cell. Figure 10 shows a group of epidermal cells drawn in the plane of the upper outer surface. Intercellular hyphae from a lower plane show through the cells. The hypha is clearly below the nucleus of the lower cell; the tip is also below the cytoplasm which seems to surround it.

I am not prepared to check Dufrenoy's observations upon other rusts

and other fungi. My own studies of *Puccinia Sorghi* are conclusive, I believe, for invagination by haustoria but for this rust Dufrenoy gives only one figure which shows haustoria. This is a tangential view of corn cells showing a late stage of the rust with heavily encased haustoria and with inconclusive evidence, in my opinion, for either plasmolysis or invagination. The other fungi upon which Dufrenoy reports I have not studied. However, I feel that the discrepancies which I have noted in the case of *Uromyces Caladii*, the fungus of his major studies, should throw doubt upon Dufrenoy's general conclusions as to host-parasite relations.

I believe that *Uromyces Caladii*, like the other rust fungi, is a highly adapted parasite in that it neither penetrates nor plasmolyses the host cytoplasm and by its invagination makes little appreciable alteration in the physical condition of the cytoplasm.

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Explanation of plates

The figures were drawn with the aid of a camera lucida. A Spencer apochromatic objective 4 mm. and ocular 10× were used, with approximate magnification ×650.

The material for figure 3 was fixed in Flemming's medium solution, cut 7.5 microns thick, and stained in Flemming's triple stain. The other figures were drawn from strips of epidermis mounted in 8% sugar solution. Figures 1, 2, 6, 7–10 were stained in 10% neutral red; figures 4 and 5 were unstained.

Plates 6–8

Uromyces Caladii in leaves of *Arisaema triphyllum*.

Fig. 1. Tangential view of cells of the lower epidermis which was mounted with the outer side uppermost

Fig. 2. An epidermal cell from the same mount as that of figure 1 after evaporation had so condensed the mounting fluid as to cause plasmolysis.

Fig. 3. A view from a cross section of a leaf showing intercellular hyphae in a space between the lower epidermis and the spongy parenchyma.

Fig. 4. Tangential view of cells of the lower epidermis which was mounted inner side uppermost.

Fig. 5. A higher plane of the right hand area of figure 4 showing the spongy parenchyma and a hypha which lies in the space between parenchyma and epidermis.

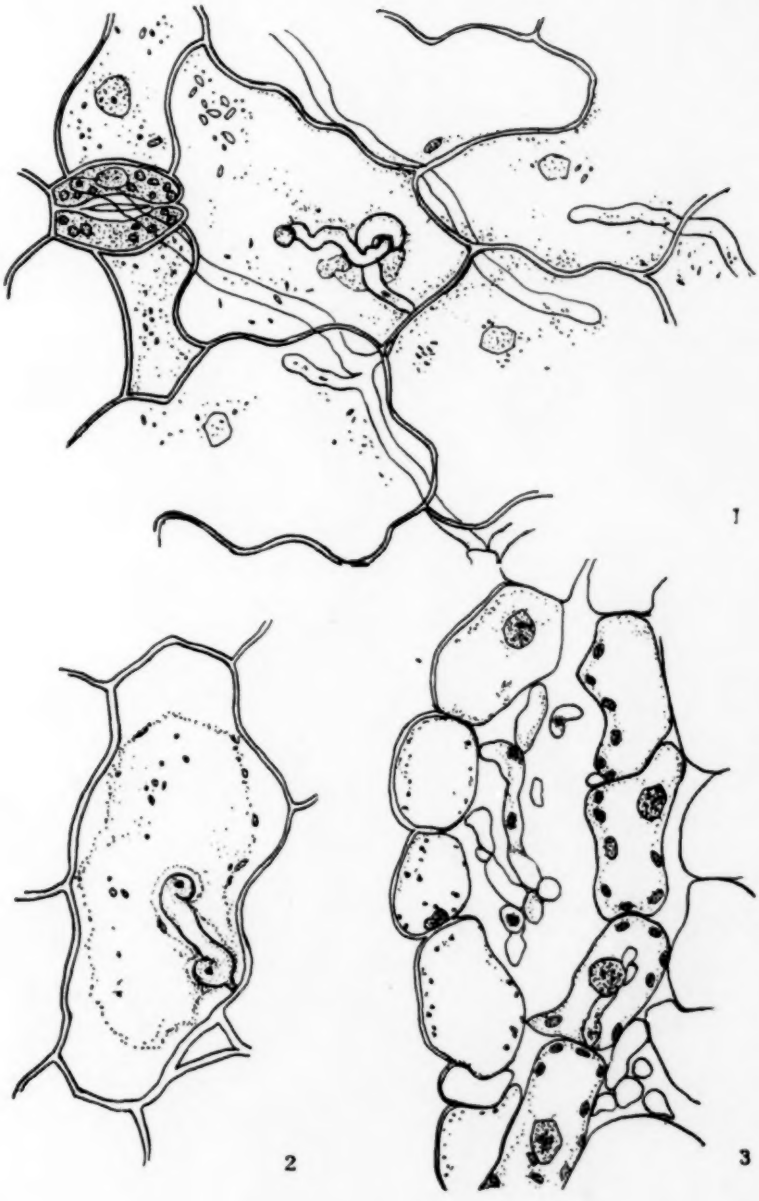
Fig. 6. Tangential view of an epidermal cell in the plane of the slender neck of a haustorium.

Fig. 7. Tangential view of a cell of lower epidermis which was mounted inner side uppermost. An intercellular hypha lying above the cell shows faintly.

Fig. 8. A view of the same cell that is shown in figure 7 in the higher plane of the intercellular hypha.

Fig. 9. Tangential view of epidermal cells which were mounted inner side uppermost showing an overlying intercellular hypha.

Fig. 10. Tangential view of cells of lower epidermis which was mounted outer side uppermost. An intercellular hypha which lies at a lower level shows through the cells.



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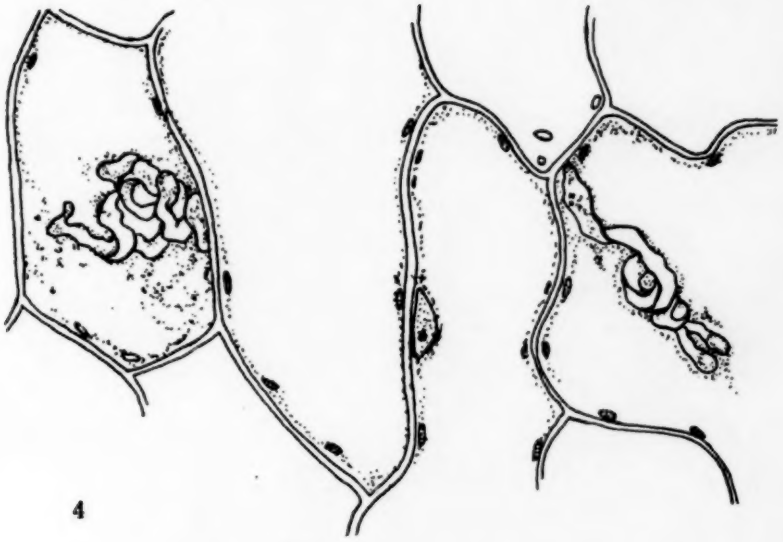

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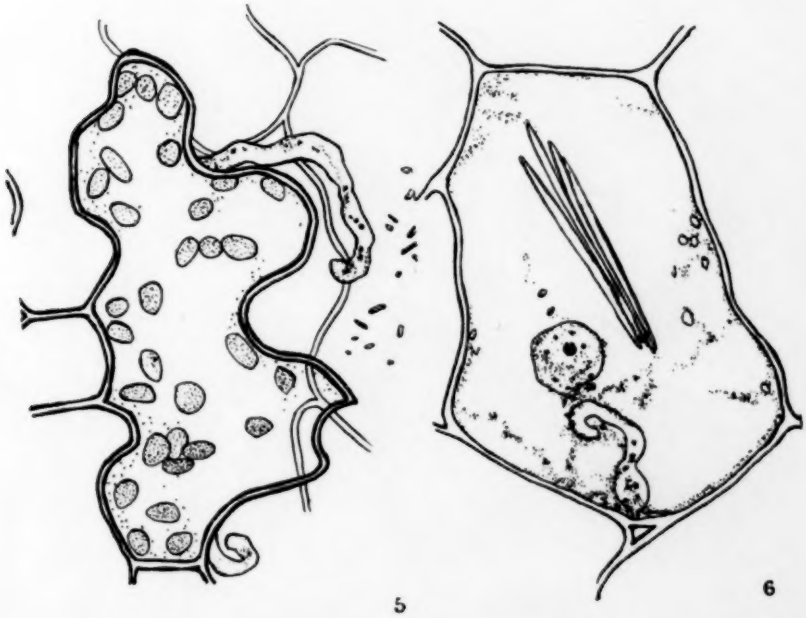

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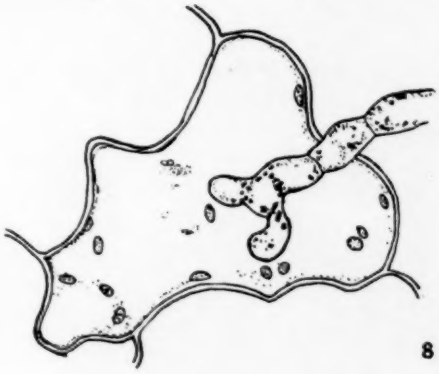

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INDEX TO AMERICAN BOTANICAL LITERATURE
1930–1933

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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